Modification of the Side Chain of Fusidic Acid (Ramycin)

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Received September 24, 1966

Treatment of fusidic acid with an equimolar amount of ozone produced the 24-aldehyde **2a** and a small amount of the 17-ketone **10**. The structure of the aldehyde **2a** was proved by transformation into a nitrile **7**. The aldehyde function of **2a** was reduced to an alcohol **8** and was also converted into a methyl group, compound **9**. The antibacterial activities of these products and of some derivatives were determined.

Fusidic acid (**1a**) is an antibiotic with a unique steroid structure,[1] which is highly active against several gram-positive bacteria[2] and used clinically mainly for the treatment of staphylococcal infections.[3] Fusidic acid is produced by the fungus *Fusidium coccineum*[1] and by several cephalosporiae.[4] We recently demonstrated that ramycin, an antibiotic isolated from the fermentation broth of *Mucor ramannianus*, is identical with fusidic acid.[5]

Discovery of the high antibacterial activity of fusidic acid has stimulated interest in antibiotics with a related structure such as helvolic acid and cephalosporin P_1.[6] The observation that 24,25-dihydrofusidic acid has nearly the same activity as the parent compound[7] suggested the desirability of a study of other derivatives with a modified side chain.

Chemistry.—Treatment of fusidic acid with an equimolar amount of ozone produced mainly the 24-aldehyde **2a** together with some of the 17-ketone **10** (Scheme I). Evidence of the presence of an aldehyde group in **2a** was obtained by the preparation of a thiosemicarbazone and a guanylhydrazone and in the following manner. The aldehyde acid **2a** was transformed into the methyl ester **2b**, which was also prepared by ozonization of the methyl ester of fusidic acid. The reaction of **2b** with hydroxylamine yielded a mixture of isomeric oximes (**3**), which upon treatment with acetic anhydride gave the fully acetylated nitrile **7**. The structure of **7** is based on the elemental analysis and the presence of a band at 2215 cm^{-1} in the infrared spectrum. The same nitrile could also be obtained by ozonization of the methyl ester of 3,11-diacetylfusidic acid (**4b**), and by transformation of the aldehyde **5b** into the nitrile **7** *via* the oxime **6**. The preparation of 3,11-diacetylnitrile **7** from **2** also proves that both hydroxyl groups are present in the aldehyde **2**. The isolation of **2** and **10** indicates that, contrary to previous observations,[8] fusidic acid can be ozonized without oxidation of the free hydroxyl groups.

The preparation of 3,11-diacetylfusidic acid (**4a**) by acetylation of fusidic acid with acetic anhydride–pyridine is also of some interest because of the report that the antibiotic resists complete acetylation under these conditions.[7] The structure of **4a** was proved by transformation of the acid into the known tetrahydro derivative, which has been prepared by acetylation of tetrahydrofusidic acid in the presence of an acid catalyst.[8]

The aldehyde group of **2a** was reduced to an alcohol group (**8**) with sodium borohydride and was also transformed into a methyl group **9** by reduction of the tosylhydrazone, according to a recently described method.[9]

Treatment of fusidic acid with an equimolar amount of *m*-chloroperbenzoic acid gave the monoepoxide. The epoxide is assumed to be on C-24,25 in analogy with the sequence of the hydrogenation[8] and the ozonization. This assumption was affirmed by the fact that dihydrofusidic acid reacted much more slowly with *m*-chloroperbenzoic acid than fusidic acid.

Antibacterial Activity.—All substances were examined for their *in vitro* activity against gram-positive and gram-negative bacteria. Only **2a** and **9** showed significant activity, with a minimum inhibitory concentration against *Staphylococcus aureus* of, respectively, 5 and 0.3 μg/ml, compared with 0.05 μg/ml for fusidic acid. All other compounds were inactive at a concentration of 10 μg/ml. Certain derivatives of **2a** revealed some tuberculostatic activity, with a minimum inhibitory concentration against *Mycobacterium tuberculosis* of 0.5 μg/ml for the guanylhydrazone and 0.3 μg/ml for the thiosemicarbazone, compared with 0.1 μg/ml for fusidic acid.

Experimental Section

Melting points were determined on a Büchi–Tottoli melting point apparatus and are uncorrected. Specific rotations, unless otherwise noted, were measured in $CHCl_3$ (*c* 1) and at 20–25°. Infrared spectra were recorded with a Beckman spectrophotometer model IR 4. Microanalyses were performed by A. Bernhardt, Mülheim, Germany. Thin layer chromatograms were run on plates coated with 0.25-mm layers of silica gel G (Merck, for thin layer chromatography). The spots were developed by spraying with a saturated solution of $SbCl_3$ in $CHCl_3$. Column chromatographic separations were made with use of silica gel (Merck, for chromatography, less than 0.08 mm) packed in toluene when elution was performed with the toluene–acetic acid–water solvent system and in benzene for the benzene–ethyl acetate mixtures. The eluates were collected in 10-ml fractions. The course of the column was checked by thin layer chromatography.

For determination of the *in vitro* activity against *Staphylococcus aureus* 6538 P the agar dilution method was used: different concentrations of the substances were incorporated in the medium containing nutrient agar (Difco B 1) and 0.5% dextrose, and an inoculum of a 24-hr growth, containing approximately 10^6 microorganisms/ml, was streaked on the surface. Inhibition was noted after 20 hr at 37° by visual inspection. For *M. tuberculosis* $H_{37}Rv$, Dubos medium (Difco B 291) was used;

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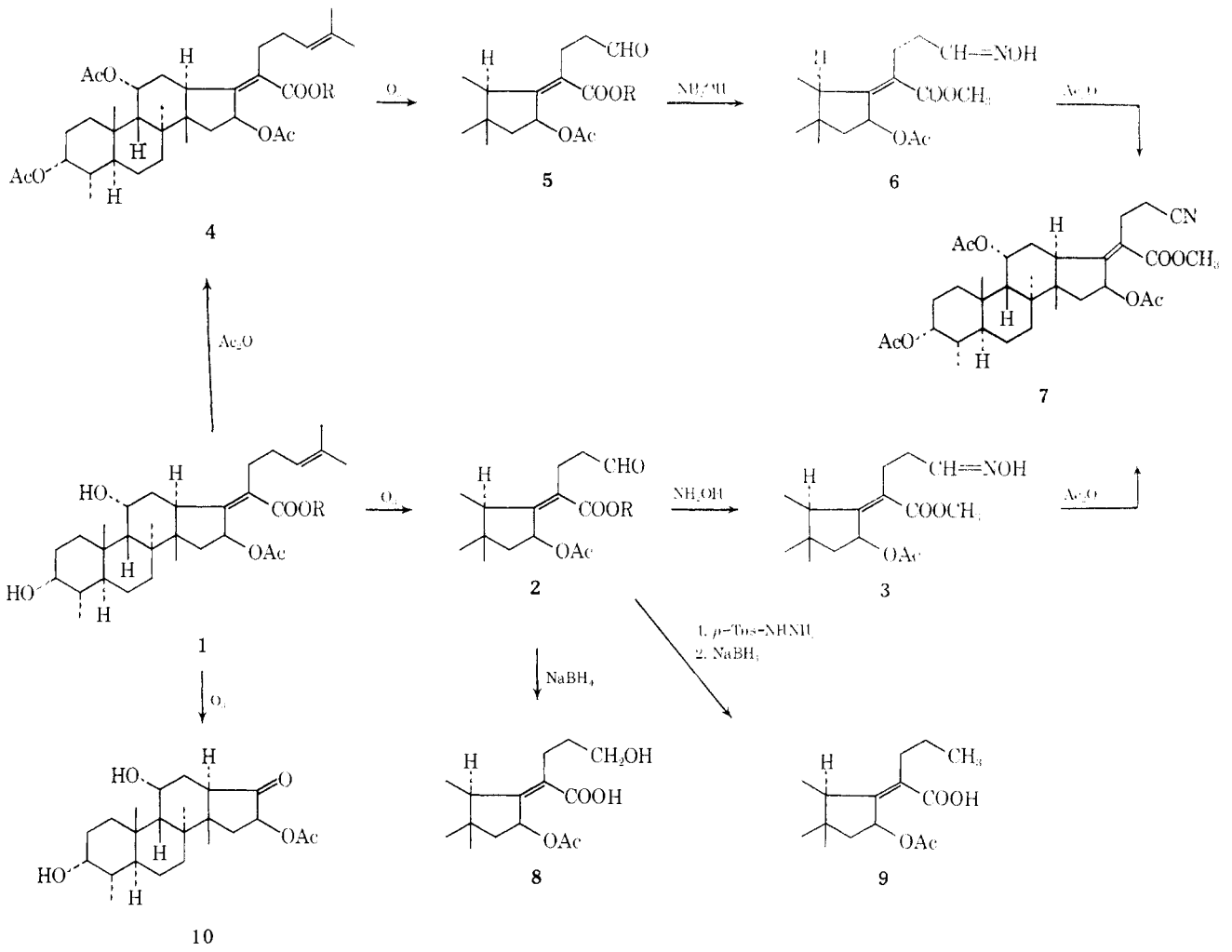
SCHEME I



a 6-day-old culture was taken as inoculum. The inhibition was observed after 4 days.

Partial Ozonization of Fusidic Acid. Method A.—Ozonized oxygen containing 2 mmoles of ozone was passed through a solution of 1034 mg (2 mmoles) of fusidic acid in 50 ml of dry CH_2Cl_2 containing 0.324 ml of dry pyridine, which was cooled to −75° in an acetone–Dry Ice bath. After addition of 660 mg of zinc dust and 1.32 ml of acetic acid, the solution was warmed to 20°, whereupon the white precipitate, which was formed during ozonization, dissolved. The reaction mixture was stirred for 2 hr, filtered from the precipitate, and washed with water. Removal of the solvent and drying of the residue yielded 980 mg of crude aldehyde **2a**, which could not be obtained in crystalline form.

Method B.—A solution of 775 mg (1.5 mmoles) of fusidic acid in 7.5 ml of methanol and 7.5 ml of ethyl acetate was cooled to −75° in an acetone–Dry Ice bath. Ozonized oxygen containing 1.5 mmoles of ozone was passed through the solution. To the cold solution was added 150 mg of 5% Pd–$CaCO_3$, and the ozonide was hydrogenated under atmospheric pressure. In 15 min 45 ml of hydrogen was taken up and absorption then ceased. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The residual oil was dissolved by shaking with 20 ml of ether containing 2 ml of CH_2Cl_2 and 5 ml of water. The water layer was discarded and the ethereal solution was washed with water. Evaporation of the solvent and drying of the residue gave 650 mg of crude material. This was dissolved in the upper phase of the solvent system toluene–acetic acid–water (5:5:1) and the solution was applied to a column of 35 g of silica gel. Elution with the same solvent system yielded **10** as the first product. The dried residue (80 mg) was recrystallized from heptane–ether and gave 50 mg of **10**: mp 182–185°; $[\alpha]D$ −113°; infrared absorption ($CHCl_3$) at 1763 (C=O), 1750 (ester C=O), and 1255 cm^{-1} (acetate).

Anal. Calcd for $C_{25}H_{36}O_5$: C, 70.37; H, 9.24; CH_3CO, 10.96. Found: C, 70.55; H, 9.21; CH_3CO, 10.71.

The fractions containing **2a**, obtained on further elution, were evaporated. The residue was taken up in methylene chloride. Some insoluble material was removed by filtration, and the solvent was evaporated. The residue was dried to yield 420 mg of amorphous **2a**: infrared absorption (KBr) at 1731 (broad) and 1260 cm^{-1} (acetate). A satisfactory elemental analysis could not be obtained. Thin layer chromatography with the upper phase of the solvent system toluene–acetic acid–water (5:5:1) showed that the products obtained by these different methods were identical. The identity of these compounds was also proved by their conversion into the methyl ester oximes followed by dehydration with acetic anhydride to yield the same acetylated nitrile, as shown by thin layer chromagography and mixture melting point (see below).

Thiosemicarbazone and Guanylhydrazone of 2a.—Compound **2a** was converted to the thiosemicarbazone by refluxing equimolar amounts of the aldehyde and thiosemicarbazide in aqueous ethanol for 30 min. After dilution with water the solid precipitate was filtered off, washed with water, and recrystallized from aqueous methanol. The purified substance decomposed at 172°, $[\alpha]D$ −14° (absolute ethanol).

Anal. Calcd for $C_{29}H_{45}N_3O_6S$: C, 61.78; H, 8.04; N, 7.45. Found: C, 61.66; H, 8.14; N, 7.34.

The guanylhydrazone of **2a** was prepared by refluxing equimolar amounts of **2a** and aminoguanidine bicarbonate in ethanol for 30 min. After cooling, the reaction mixture was poured into ether and the resulting precipitate was filtered off and dried. Treatment with active charcoal and recrystallization from

aqueous ethanol afforded the pure guanylhydrazone, which decomposed at 204°.

Anal. Calcd for $C_{29}H_{46}N_4O_6 \cdot 0.5H_2O$: C, 62.67; H, 8.52; N, 10.08. Found: C, 62.90; H, 8.42; N, 9.88.

Synthesis of the Aldehyde Ester 2b. Method A.—A solution of 2.12 g (4 mmoles) of fusidic acid methyl ester and 0.324 ml of dry pyridine in 50 ml of dry CH_2Cl_2 was cooled to −75° in an acetone–Dry Ice bath, and ozonized oxygen containing 4 mmoles of ozone was passed through the solution. After cleavage of the ozonide with 1.32 g of zinc dust and 2.64 ml of acetic acid, the reaction mixture was worked up as described for the preparation of **2a**, yielding 1.9 g of the crude aldehyde. This was purified by adsorption from benzene on 100 g of silica gel and elution with benzene–ethyl acetate (1:1). The fractions containing pure **2b** were combined and evaporated. The residue was dissolved in CH_2Cl_2, filtered, evaporated, and dried to yield 1.2 g of **2b** as amorphous material, which could not be crystallized.

Anal. Calcd for $C_{29}H_{44}O_7$: C, 69.02; H, 8.79. Found: C, 68.57; H, 9.18.

Method B.—One gram of **2a** dissolved in CH_2Cl_2 was esterified with ethereal diazomethane. After removal of the solvent the residue was purified by adsorption chromatography on 50 g of silica gel, as described in method A, yielding 400 mg of amorphous **2b**. The identity of the products obtained by methods A and B was proved by thin layer chromatography with benzene–ethyl acetate (1:3) and by the fact that the acetylated nitriles obtained by dehydration of their oximes were identical (mixture melting point and thin layer chromatography; see below).

3,11-Diacetylfusidic Acid (4a).—A solution of 10 g of fusidic acid in a mixture of 20 ml of dry pyridine and 20 ml of acetic anhydride was heated in an oil bath at 100–105° for 16 hr. The reaction mixture was cooled and was poured slowly with stirring into 250 ml of ice water. Stirring was continued for 30 min. The dark viscous oil was taken up in ether, and the ethereal solution was filtered, washed with dilute HCl and water, and dried (Na_2SO_4). Ether was evaporated and the residue (10 g) was purified by adsorption chromatography of its solution in toluene on 150 g of silica gel. The column was eluted with the upper phase of the solvent system toluene–acetic acid–water (5:1:1). The fractions containing relatively pure acid were combined and evaporated under reduced pressure. The residue was dried and recrystallized from heptane–ether to yield 7.4 g of **4a**, mp 170–171°, $[\alpha]D$ −21°.

Anal. Calcd for $C_{35}H_{52}O_8$: C, 69.96; H, 8.72. Found: C, 69.90; H, 8.89.

3,11-Diacetylfusidic Acid Methyl Ester (4b).—To a solution of 0.5 g of 3,11-diacetylfusidic acid in ether was added an ethereal solution of diazomethane until the yellow color persisted. Removal of the solvent and recrystallization of the oily residue from ethanol–water yielded 0.4 g of **4b**, mp 138–139°, $[\alpha]D$ −27°.

Anal. Calcd for $C_{36}H_{54}O_8$: C, 70.32; H, 8.85. Found: C, 69.96; H, 8.80.

Synthesis of the Aldehyde Acid 5a.—Compound **5a** was prepared by the same ozonization procedures as described for the synthesis of **2a** from fusidic acid.

Method A.—Ozonization of 1.2 g (2 mmoles) of 3,11-diacetylfusidic acid followed by cleavage of the ozonide with zinc dust and acetic acid yielded 1.15 g of crude **5a**. Recrystallization from heptane–ether gave 0.6 g of pure aldehyde: mp 196° dec; infrared ($CHCl_3$), 1735 (ester C=O), 1718 sh (aldehyde C=O), 1695 sh (α,β-unsaturated acid C=O), and 1260 cm^{-1} (acetate). A satisfactory analysis could not be obtained.

Method B.—Ozonization of 3,11-diacetylfusidic acid (1.2 g) followed by reductive work-up with hydrogen in the presence of 5% Pd–$CaCO_3$ gave 1050 mg of crude aldehyde. This was recrystallized from heptane–ether to yield 500 mg of **5a**, mp 191° dec. On thin layer chromatography with the upper phase of the solvent system toluene–acetic acid–water (5:5:1) both products coincided. The semicarbazone of **5a** was prepared by refluxing **5a** with a small excess of semicarbazide hydrochloride and sodium acetate in aqueous ethanol for 2 hr. The reaction mixture was cooled, water was added, and the resulting glassy product crystallized on trituration after addition of ether. After removal of ether the crystals were filtered off, dried, and recrystallized from chloroform–acetone. The purified semicarbazone melted at 180° dec.

Anal. Calcd for $C_{33}H_{49}N_3O_9$: C, 62.73; H, 7.81; N, 6.65; O, 22.79. Found: C, 62.72; H, 7.60; N, 6.61; O, 22.79.

Synthesis of the Aldehyde Ester 5b. Method A.—Partial ozonization of 1.85 g (3 mmoles) of 3,11-diacetylfusidic acid methyl ester according to the procedure described for the synthesis of **2b** yielded 1.75 g of crude aldehyde. This material was dissolved in benzene and was chromatographed on a column of 85 g of silica gel. Elution with benzene–ethyl acetate (4:1) yielded 1.2 g of pure material. Recrystallization from heptane–ether gave 1.0 g of **5b**, mp 153–155.5° dec, $[\alpha]D$ −13°.

Anal. Calcd for $C_{33}H_{48}O_9$: C, 67.32; H, 8.21; O, 24.46. Found: C, 67.67; H, 8.23; O, 24.14.

Method B.—To a solution of 1 g of crude **5a** in ether was added an ethereal solution of diazomethane until the yellow color persisted. After removal of the solvent the residue was dissolved in benzene and this solution was poured over a column of 50 g of silica gel. Elution with benzene–ethyl acetate (9:1) yielded, after drying, 400 mg of crude ester. Upon recrystallization from heptane–ether 300 mg of pure **5b** was obtained, mp 151–154° dec, undepressed by admixture with the product obtained by method A. Thin layer chromatography with benzene–ethyl acetate (3:2) also proved the identity of both products.

Synthesis of 7. Method A. From 5b.—To a solution of 589 mg (1 mmole) of **5b** in 3 ml of ethanol was added a solution of 73 mg of hydroxylamine hydrochloride and of 146 mg of sodium acetate in 1.5 ml of water. The resulting solution was refluxed on a water bath for 30 min. Ethanol was evaporated and the residual oily suspension was shaken with ether and water. The ethereal solution was washed with water and the solvent was removed. The dried residue (600 mg) consisted of a mixture of both isomeric oximes **6**, as revealed by thin layer chromatography with benzene–ethyl acetate (3:2), which showed the presence of two products. The analytical sample of this mixture was prepared by adsorption on silica gel and elution with benzene–ethyl acetate (3:2).

Anal. Calcd for $C_{33}H_{49}NO_9$: C, 65.64; H, 8.18; N, 2.32. Found: C, 65.98; H, 8.40; N, 2.31.

A solution of 600 mg of the mixture of the isomeric oximes **6** in 6 ml of acetic anhydride was heated on a water bath for 15 hr. Most of the solvent was evaporated under reduced pressure and the viscous residue was shaken with water and a small amount of ether for 1 hr. More ether was added and the ethereal solution was washed successively (H_2O, 5% $NaHCO_3$, H_2O). Upon removal of the solvent and recrystallization of the residue from heptane–ether, 350 mg of **7**, mp 165–166°, was obtained: $[\alpha]D$ −34°; infrared ($CHCl_3$), 2215 (C≡N), 1733 (ester C=O), 1721 sh (α,β-unsaturated ester C=O), and 1256 cm^{-1} (acetate).

Anal. Calcd for $C_{33}H_{47}NO_8$: C, 67.66; H, 8.09; N, 2.39. Found: C, 67.41; H, 7.93; N, 2.40.

Method B. From 2b.—By the same procedure, 505 mg (1 mmole) of **2b** was converted into a mixture composed of both isomeric oximes **3**, as assumed from the presence of two products on a thin layer chromatogram developed with benzene–ethyl acetate (1:3). The yield was 500 mg. The analytical sample of this mixture was prepared by adsorption on silica gel and elution with benzene–ethyl acetate (1:1).

Anal. Calcd for $C_{29}H_{43}NO_7$: C, 67.02; H, 8.73; N, 2.69. Found: C, 66.75; H, 8.82; N, 2.51.

A solution of 500 mg of **3** in 5 ml of acetic anhydride was heated on a water bath for 15 hr. The mixture was processed as for the dehydration of **6**. Recrystallization of the residue gave 300 mg of **7**, mp 164–165°; no depression was observed in admixture with a sample obtained by method A. On thin layer chromatography with benzene–ethyl acetate (3:2), a mixture of both compounds showed only one spot.

Reduction of 2a to 8.—To an ice-cooled solution of 370 mg of $NaBH_4$ in a mixture of 5 ml of water and 5 ml of methanol was added dropwise with stirring a solution of 982 mg (2 mmoles) of **2a** in a mixture of 5 ml of methanol and 2 ml of water, neutralized with 5% aqueous $NaHCO_3$. The resulting solution was first stirred for 15 min at 0° and then for 15 min at room temperature. After dilution with 50 ml of water and addition of 50 ml of a mixture of ether–CH_2Cl_2 (2:1) the mixture was cooled in ice water and acidified with dilute HCl. The water layer was discarded and the organic solution was washed with water until neutral and then evaporated. Recrystallization of the residue from aqueous methanol yielded 400 mg of **8**: mp 156–157.5° dec; $[\alpha]D$ +5° (absolute ethanol); infrared (KBr), 1740 (ester C=O), 1694 (α,β-unsaturated acid C=O), and 1248 cm^{-1} (acetate).

Anal. Calcd for $C_{28}H_{44}O_7$: C, 68.26; H, 9.00; CH_3CO, 8.73. Found: C, 68.46; H, 8.97; CH_3CO, 8.57.

Conversion of 2a into 9.—A solution of 491 mg (1 mmole) of **2a** and 186 mg (1 mmole) of *p*-tosylhydrazide in 5 ml of methanol was refluxed for 30 min. The solvent was evaporated; after drying, 640 mg of crude hydrazone was obtained. A solution of 659 mg (1 mmole) of this compound in a mixture of 5 ml of methanol and 2 ml of water was neutralized with 5% aqueous $NaHCO_3$, and the resulting solution was added with stirring to an ice-cooled solution of 740 mg of $NaBH_4$ in a mixture of 5 ml of methanol and 5 ml of water. The reaction mixture was first stirred for 15 min at 0° and then for 15 min at room temperature. After dilution with water the mixture was acidified with dilute HCl. The precipitate was filtered off and washed with water until neutral. The dried residue (420 mg) was recrystallized from aqueous methanol; 150 mg of pure **9**, mp 202–204° dec, was obtained; $[\alpha]D$ +7° (absolute ethanol); infrared (KBr), 1745 (ester C=O), 1701 (α,β-unsaturated acid C=O), and 1256 cm^{-1} (acetate).

Anal. Calcd for $C_{28}H_{34}O_6$: C, 70.55; H, 9.30; CH_3CO, 9.03. Found: C, 71.10; H, 9.23; CH_3CO, 8.94.

Methyl Ester of 9.—To a suspension of 750 mg of **9** in ether was added with stirring an ethereal solution of CH_2N_2 until the yellow color persisted. The resulting solution was evaporated, and the residue was recrystallized from aqueous methanol, yielding 620 mg of pure ester: mp 160.5–162°; $[\alpha]D$ +1°; infrared ($CHCl_3$), 1737 (ester C=O), 1723 sh (α,β-unsaturated ester C=O), and 1264 cm^{-1} (acetate).

Anal. Calcd for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.80; H, 9.33.

3-Acetate of 9.—A solution of 0.5 of **9** in a mixture of 2.5 ml of acetic anhydride and 2.5 ml of pyridine was kept at room temperature for 15 hr. The reaction mixture was treated, as usual, to yield 0.5 g of crude 3-acetate. After recrystallization from aqueous methanol the purified material (0.45 g) softened at 141° and melted at 157–158°, $[\alpha]D$ −17°.

Anal. Calcd for $C_{30}H_{48}O_7 \cdot H_2O$: C, 67.13; H, 9.01. Found: C, 67.24; H, 8.96.

24,25-Oxidofusidic Acid.—A solution of 2.58 g (5 mmoles) of fusidic acid in 125 ml of $CHCl_3$ was treated with 1 equiv of *m*-chloroperbenzoic acid dissolved in 25 ml of $CHCl_3$. After standing for 30 min at room temperature, titration and thin layer chromatography of the reaction mixture showed that the reaction was complete. After removal of the solvent under reduced pressure, the residue was dissolved in CH_2Cl_2 and added to hot heptane. Methylene chloride was allowed to evaporate and heptane was decanted from the residue, which was then extracted once more in a similar manner. The residue was dried and yielded 2.5 g of product which could not be obtained in a crystalline form.

Upon a similar epoxidation using dihydrofusidic acid instead of fusidic acid, titration of an aliquot part of the reaction mixture showed that the peracid content was not yet decreased by 10% after 30 min.

24,25-Oxidofusidic Acid Methyl Ester. Method A.—The reaction of fusidic acid methyl ester with *m*-chloroperbenzoic acid was carried out as described for fusidic acid. When reaction was complete the solvent was evaporated under reduced pressure and the residue was taken up in ether. The ethereal solution was washed ($NaHCO_3$, H_2O) and evaporated. From 1.33 g of fusidic acid methyl ester, 1.45 g of crude oxido ester was obtained; after chromatography on 75 g of silica gel, elution with benzene–ethyl acetate (2:3) yielded 1 g of pure product which failed to crystallize; $[\alpha]D$ −11°.

Anal. Calcd for $C_{32}H_{50}O_7$: C, 70.29; H, 9.22. Found: C, 70.05; H, 9.24.

Method B.—To a solution of 500 mg of crude 24,25-oxidofusidic acid in ether was added an ethereal solution of CH_2N_2 until the yellow color persisted. After removal of the solvent the residue (500 mg) was chromatographed on 25 g of silica gel and was eluted with benzene–ethyl acetate (2:3) to yield 250 mg of pure 24,25-oxidofusidic acid methyl ester. The identity of this compound with the compound obtained by method A was checked by thin layer chromatography with benzene–ethyl acetate (2:3).

Acknowledgments.—We thank Mr. P. Van Dijck for the determination of the antibacterial activities and Mr. J. Roudelet for the infrared spectra.

Anthelmintic Activity of 1,2,4-Oxadiazoles

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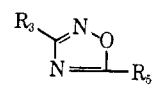
Received June 18, 1966

Substituted 1,2,4-oxadiazoles were screened for anthelmintic activity in mice experimentally infected with *Nematospiroides dubius*. 3-Alkyl- and 3-aryl-1,2,4-oxadiazoles without substituents at the 5 position were effective when administered orally at 500 mg/kg or by subcutaneous injection at 100 mg/kg, whereas the 5-substituted 3-alkyl- and 3-aryl-1,2,4-oxadiazoles tested were inactive. 3-*p*-Chlorophenyl-1,2,4-oxadiazole was chosen for extended studies against nematode infections in experimental animals. 5-Spiro-4,5-dihydro-1,2,4-oxadiazoles were prepared.

Recently, 1,2,4-oxadiazoles have received considerable attention in the literature.[2,3] Substituted 1,2,4-oxadiazoles have been reported to exhibit various types of biological activities, including antispasmodic and analgetic,[4] sedative,[5] and nematocidal, fungicidal, and insecticidal.[6] We wish to report our finding that 3-substituted 1,2,4-oxadiazoles are anthelmintics when tested against *Nematospiroides dubius* in mice.

Preliminary screening of 1,2,4-oxadiazoles of type I, wherein R_3 and R_5 are hydrogen, alkyl, or aryl, indicated that the anthelmintic activity was considerably



I

better for 3-substituted 5-hydrogen-1,2,4-oxadiazoles than for any other combination of 3 and 5 substitution. The 3-substituted 1,2,4-oxadiazoles that were evalu-

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