OXIDATION OF FUSIDIC ACID

G. S. Suchkova, G. B. Lokshin,
A. D. Kuzovkov, and A. I. Chernyshev

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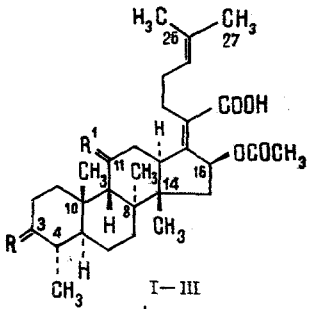
When the antibiotic fusidic acid (I) was oxidized with chromium trioxide, only 3,11-dioxofusidic acid (II) was isolated [1]. With the aim of the possible identification of other products and the inactivation of the antibiotic, we have performed its oxidation under the conditions described previously [1]. When the products of the reaction were chromatographed on silica gel, in addition to (II) we isolated a compound (III), the structure of which we established as 11-oxofusidic acid on the basis of the facts given below.



I–III

I. R=R'=H, α-OH
II. R=R'=O
III. R=H, α-OH, R'=O

Compound (III) corresponds to the empirical formula $C_{31}H_{46}O_6$, which differs from the empirical formula of (I) ($C_{31}H_{48}O_6$) by two hydrogen atoms. The ^{13}C NMR spectrum of substance (III) (see below) has a signal at δ 209.8 ppm corresponding in position and intensity to the carbon of a carbonyl group, and, therefore, (III) is a keto derivative of fusidic acid. Below we give the chemical shifts of the signals of the carbon atoms in the ^{13}C NMR spectra of fusidic and monoxo- and dioxofusidic acids*:

Compound	δ, ppm		
	C_3	C_{11}	$COCH_3$
Fusidic acid (I)	71.4	68.1	170.4
Monooxofusidic acid (III)	71.3	209.8	170.2
Dioxofusidic acid (II)	215.2	209.1	170.0

When compound (I) was oxidized, the O–Ac group at C_{16} was not split off (this reaction takes place under very mild conditions for fusidic acid), since the ^{13}C NMR spectra of compounds (II) and (III) retained the signal of the carbon of a carbonyl group in a $COCH_3$ residue ($\delta \sim 170$ ppm).

As was to be expected, on passing from fusidic acid to its 3,11-dioxo derivative the signals of the carbons of two keto groups appeared (δ 209.1 and 215.2 ppm), the position of one of which was identical with that of the signal of the carbon of the C = O group in (III).

The spectrum of (III) retained the signal of the carbon of a C – OH group (δ 71.3 ppm), and, consequently, this compound is a monooxo acid. However, it is not clear from the ^{13}C NMR spectra which of the atoms – C_3 or C_{11} – is involved in the oxo group in (III). It is possible to determine the position of the oxo group in (III) by comparing the ^1H NMR spectra of compounds (I–III).

The chemical shifts (δ, ppm) of the signals of the protons of the methyl groups in the ^1H spectra of fusidic and monooxo- and diketofusidic acids are as follows:

* The assignment of the C_3 and C_{11} signals was made on the basis of the ^1H and ^{13}C NMR spectra.

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Compound	δ, ppm			
	CH_3 at C_4	C_8*,	C_{10},	C_{14}
Fusicid acid (I)	0.92 J=7.2 Hz	0.92	0.98	1.38
11-Oxofusidic acid (III)	0.91 J=7.2 Hz	1.02	1.18	1.18
3,11-Dioxofusidic acid (II)	1.05 J=7.2 Hz	1.06	1.15	1.21

*No assignment of the signals to individual CH_3 groups was made.

The chemical shift of the doublet signal of the protons of the methyl group at C_4 scarcely varies in the spectra on passing from (I) to (III), while in dioxofusidic acid this signal is shifted downfield by 0.13 ppm, which shows the position of the oxo group in (III) in a part of the molecule more remote from C_4. This conclusion is confirmed by the influence of the 11-oxo group on the positions of the signals of the protons of the neighboring methyls (at C_8, C_{10}, and C_{14}) in the spectra of (II) and (III), which are shifted by 0.1-0.2 ppm in comparison with the corresponding signals in the spectra of fusidic acid. Thus, in (III) the oxo group is present at C_{11}.

11-Oxofusidic acid retains some activity in relation to Gram-positive bacteria in vitro, but does not act on strains resistant to fusidic acid. The antibacterial spectrum of 11-oxofusidic acid is as follows:

Test organism	MSA, μg/ml
Staphylococcus aureus 209P	0.78
Staphylococcus aureus 209P resistant to fusidic acid	>200
Streptococcus faecalis	6.25
Sarcina lutea	1.56
Bac. mycoides	6.25
Bac. subtilis	3.12
E. coli	>200
Pseudomonas acruginosa	>200

Up to the present, there has been no information in the literature on the production or isolation of 11-oxofusidic acid from natural sources.

EXPERIMENTAL

For column chromatography we used L 40/100 μ silica gel (Czechoslovakia). Thin-layer chromatography was performed on Silufol plates in chloroform – ethyl acetate – methanol (7 : 2 : 1). The revealing agent was I_2 vapor. The results of the elementary analysis of the compounds obtained corresponded to the calculated figures. The IR spectra of the samples in tablets with KBr were recorded on a UR-20 spectrophotometer. The ^1H NMR (90 MHz) and ^{13}C NMR (22.624 MHz) spectra were recorded at 40°C on a WH-90 pulse radiospectrometer using 4% and 10% solutions, respectively, of the substances in $CDCl_3$. The chemical shifts of the NMR spectra are given in the δ scale. Tetramethylsilane (^1H NMR) and $CDCl_3$ (^{13}C NMR) were used as internal standards. For the ^{13}C NMR spectra the chemical shifts were recalculated to the δ scale using the relation $\delta_{CDCl_3} = 76.9$ ppm.

11-Oxofusidic Acid (III). With stirring at 5°C, 16 ml of Kiliani's reagent was added in portions over 10 min to a solution of 10 g of chromatographically pure fusidic acid in 200 ml of acetone that had been freshly distilled over $KMnO_4$. After the end of oxidation, 300 ml of water was added to the reaction mixture and the acetone was distilled off in vacuum.

The aqueous suspension was extracted with ether (2 × 70 ml), and the combined extract, after washing with water and drying over Na_2SO_4, was evaporated to dryness, and the residue was then chromatographed on a column. Elution was performed with chloroform and with chloroform – ethyl acetate (9.5 : 0.5). The eluates were analyzed by TLC. This showed that the chloroform eluate contained only 3,11-dioxofusidic acid, which was obtained from the residue after evaporation of the solvent by crystallization from ether. Weight 1.8 g, mp 193-194°C (according to the literature: 197-198°C [1]).

Chloroform – ethyl acetate eluted compound (III), which was isolated from the residue after evaporation of the eluate by precipitation with hexane. Yield 1.3 g, mp 121-123°C (decomp.); $[\alpha]_D^{20}$ +49.8° (c 1.00; chloroform); ν_{max}^{KBr} 3400-3420 cm^{-1} (OH), 1740-1690 cm^{-1} (COOH, $COCH_3$, CO), 1260 cm^{-1} (C–OH). ^1H NMR spectrum: 0.91 (d, J = 7.2 Hz, CH_3 at C_4); 1.02, 1.18 (s, 3 H, and s, 6 H, respectively; CH_3 at C_8, C_{10}, C_{14}); 1.59 (s, 3 H, CH_3-26); 1.67 (s, 3 H, CH_3-27), 1.98 (s, 3 H, CH_3COO at C_{16}), 3.76 (s, H at C_3). ^{13}C NMR spectrum, ppm; 71.3 (C_3); 74.1 (C_{16}); 170.2 ($\underline{CO}CH_3$); 209.8 (C_{11} = O).

SUMMARY

11-Oxofusidic acid – a previously unknown keto derivative of the antibiotic – has been isolated from the products of the oxidation of fusidic acid by chromium trioxide.

LITERATURE CITED

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STUDY OF THE FRACTIONAL COMPOSITION OF THE DIOXANE LIGNIN FROM THE COTTON PLANT

N. A. Veksler, M. S. Rakhmatullaev, L. S. Smirnova, and Kh. A. Abduazimov

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It has been shown previously [1] that the dioxane lignin (DLA) from ripe cotton-plant stems is polydisperse. For a detailed study we have fractionated it in terms of molecular weights by successive precipitation from dioxane solution with ether by the triangle method [2]. This method gives a small number of fractions, but each of them has a comparatively narrow molecular-weight distribution. The separation yielded six fractions (I-VI) with successively decreasing molecular weights, since with an increase in the volume fraction of precipitant lignin fractions with ever-smaller molecular weights precipitate. Fractions (I) and (III) were the largest.

To check the efficiency of separation and to calculate molecular weights, the fractions obtained were subjected to gel chromatography in an analytical column containing Sephadex G-75 with dimethyl sulfoxide (DMSO) as solvent and eluent. Fig. 1a shows eluograms of the fractions and, for comparison, an eluogram of the unfractionated DLA is given. From these eluograms we plotted integral curves of molecular weight distribution (MWD), using the coefficients found previously [3]. The weight-average and number-average molecular weights ($\overline{M}_w$ and $\overline{M}_n$) of the fractions were calculated from the integral MWD curves. These values and their ratios ($\overline{M}_w/\overline{M}_n$), which characterize the degree of polydispersity of the fractions, are given below:

Fraction No.	Yield, %	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
Initial DLA	100	12000	4200	2,9
I	19,8	21800	15000	1,45
II	5,8	19000	11200	1,7
III	22,5	12000	7500	1,6
IV	12,8	5700	3700	1,5
V	5,7	4100	3200	1,3
VI	12,6	3000	2200	1,4

The polydispersities of the fractions were different, varying from 1.7 to 1.3. The fractions of lowest molecular weight, (V) and (VI), were the most homogeneous. By comparing the $\overline{M}_w$ and $\overline{M}_n$ values of the fractions we can see that the selected method of fractionation is fairly effective for DLA, since the molecular weights of the fractions differ considerably.

It is known [4] that spruce DLA is inhomogeneous in relation both to molecular weight and to chemical composition. Consequently, for each fraction we performed an elementary analysis and a quantitative analysis of functional groups. The results* of the analytical investigation of the DLA fractions are given below (%):

Fraction No.	C	H	OCH_3	OH_{tot}	CO	OH_{COOH}	OH_{ph}	COOH	Carbohydrates
Initial DLA	59,94	6,40	19,52	11,02	2,92	0,48	3,38	1,27	2,80
I	58,12	6,24	18,49	11,45	2,62	0,48	2,88	1,27	3,28
II	58,22	6,40	18,40	11,58	2,58	0,49	3,40	1,30	3,40
III	58,24	6,46	19,30	11,54	3,00	0,48	3,42	1,27	3,30
IV	59,74	6,30	19,47	11,90	2,96	0,53	3,41	1,40	3,80
V	59,02	6,22	19,34	11,78	2,87	0,55	3,44	1,46	4,30
VI	57,56	6,25	18,39	12,88	4,02	0,72	3,46	1,94	8,90

*The results are given without being recalculated to carbohydrates.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

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