# THE STRUCTURE OF FERUTIN

#### A. I. Saidkhodzhaev and G. K. Nikonov

From various species of Ferula, in addition to coumarins [1-3] and sesquiterpene lactones [4-6], a group of substances consisting of esters of aromatic acids with terpenoid alcohols has been isolated [7, 8]. In the present paper we give information on the structure of one substance of this type (ferutin) which we found in a number of giant fennels growing in the Tashkent oblast.

Ferutin,  $C_{20}H_{26}O_5$ , mp 130-131°C (from petroleum ether),  $[\alpha]_D^{25} + 101.8°$  (c 1.08; CH<sub>3</sub>OH) is readily soluble in chloroform, ethanol, and ether, sparingly soluble in petroleum ether, and insoluble in water. Its UV spectrum has a maximum at 260 nm (log  $\varepsilon$  4.05), which is characteristic for an aromatic ring, and

the IR spectrum has absorption bands at (cm<sup>-1</sup>) 3300-3600 (OH), 2860-2980 (C-CH<sub>3</sub>), 1710 ( $-\ddot{C}$ -Ar), 1680 C=C) and 1610, 1585, 1515 (aromatic ring). When the UV spectrum was recorded in the presence of caustic alkali, the maximum shifted to the 310 nm region, which shows the presence in the substance of a free phenolic hydroxy group. The mass spectrum of ferutin had peaks of ions with m/e 346 (M)<sup>+</sup>, 331 (M-15)<sup>+</sup>, 328 (M-18)<sup>+</sup>, 220, 177, 168, 159, 151.

The NMR spectrum of the substance shows doublets at 7.54 and 6.93 ppm (J = 10 Hz) relating to the ortho protons of an aromatic nucleus, a one-proton singlet at 7.51 ppm, corresponding to a meta proton, signals at 5.96 and 5.50 ppm due, respectively, to a phenolic hydroxyl and an olefinic proton, a triplet with secondary splitting at 5.22 ppm ( $J_1 = 7$  Hz,  $J_2 = 3$  Hz) due to a geminal proton with respect to an ester group, and a singlet at 3.90 ppm (3H) corresponding to the protons of a methoxy group attached to an aromatic nucleus. In addition, there is a broadened signal at 2.5 ppm caused by a hydroxy group. The correctness of the latter assignment is confirmed by the results of deuterium exchange, when it disappears. In the strong-field region there are doublets from secondary methyl groups at 0.79 and 0.89 ppm (J = 7.5 Hz), from a tertiary methyl group at 1.05 ppm, and from a methyl group at a double bond at 1.77 ppm.

These facts, and also the irreversible changes of the substance after treatment with alkali show that ferutin is an ester of an aromatic acid and a terpenoid alcohol.

The alkaline hydrolysis of ferutin with 5% caustic soda solution yielded an acid  $C_8H_8O_4$  with mp 250°C, mol. wt. 168 (mass spectrometry) and an alcohol  $C_{12}H_{20}O_2$  with mp 82-83°C, mol. wt. 196 (mass spectrometry), which we have called ferutinol.

Thus, of the five oxygen atoms in ferutin, two are present in a phenolic hydroxyl and a methoxy group, two in an ester grouping, and one in an alcoholic hydroxy group.

To prove the nature of the hydroxy group, mono- and diacetates of ferutin were obtained. The acetylation of ferutin with acetic anhydride in pyridine formed a monoacetate  $C_{22}H_{28}O_6$  with M<sup>+</sup> 388 in the IR spectrum of which absorption bands appeared at 3500 cm<sup>-1</sup> (hydroxy group) and 1780 cm<sup>-1</sup> (ester carbonyl group conjugated with an aromatic nucleus). The difficulty in acetylating the second hydroxyl shows its tertiary nature. Consequently, one hydroxy group in ferutin is phenolic and the other alcoholic.

The NMR spectrum of the monoacetate has a signal at 2.17 ppm (3H) due to an acetyl group attached to an aromatic nucleus and a broadened signal at 2.25 ppm corresponding to a hydroxy group. The acetylation of ferutin with acetic anhydride in the presence of fused sodium acetate gave a diacetate,  $C_{24}H_{30}O_7$  (M<sup>+</sup> 430).

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• 1974 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$15.00. In the NMR spectrum of the latter, the signals at 5.96 ppm (HO-Ar) and 2.25 ppm (-OH) had disappeared; at the same time, a six-proton singlet had appeared at 2.17 ppm. In the IR spectrum of the diacetate there was no absorption band of a hydroxy group. The absence of the paramagnetic shift of a methine proton in the NMR spectrum of the diacetate shows that the second hydroxy group is tertiary.

The comparative constancy of the chemical shifts (CSs) of the signals of the methyl groups on passing from ferutin (0.79, 0.89, 1.05) to the diacetate (0.80, 0.90, 1.02) gives grounds for assuming that this hydroxy group is remote from the latter or, at least, is not in the geminal position.

In the hydrogenation of ferutin, one mole of hydrogen was absorbed, and dihydro derivative ( $M^+$  348) was formed.

The UV spectrum of the acid obtained on the hydrolysis of ferutin had a maximum at 260 nm, which is characteristic for a benzene ring. In the IR spectrum, absorption bands appeared at  $(\text{cm}^{-1})$  3450-3200 (hydroxy group), 1690 (carboxy group), and 1620, 1590, and 1525 (aromatic nucleus).

In the NMR spectrum of the acid in the weak-field region, there were signals of five protons of which two corresponded to the protons of carboxy and hydroxy groups (10.8 ppm, 2H), and three to aromatic protons. Doublets at 7.90 and 6.93 ppm (J = 8 Hz) related to ortho protons of an aromatic nucleus, and a broadened singlet at 8.15 ppm was due to a meta proton. In addition, a three-proton singlet was observed at 3.92 ppm, due to the protons of a methoxy group. The presence in the IR spectrum of the acid of absorption bands in the 890, 840 cm<sup>-1</sup> regions shows that the substance is based on a 1,2,4-substituted benzene ring [9]. The downfield shift of two protons (to 7.90 and 8.15 ppm) in comparison with the protons of unsubstituted benzene (7.27 ppm) is explained by the electron-accepting influence of a carboxy group in the ortho position to them. On this basis, it may be assumed that the acid obtained by the hydrolysis of ferutin is 3-hydroxy-4-methoxy- or 4-hydroxy-3-methoxybenzoic acid.

The acetylation of the acid with acetic anhydride in pyridine gave a monoacetate  $C_{10}H_{10}O_5$  with mp 212-213°C in the NMR spectrum of which there was a three-proton singlet at 2.17 ppm relating to an acetyl group in the aromatic nucleus. The IR spectrum showed an absorption band at 1780 cm<sup>-1</sup>, which is characteristic for a carbonyl group conjugated with an aromatic nucleus.

On comparing the physicochemical constants of the acid and its acetate with literature data, it was seen that it was identical with isovanillic acid (3-hydroxy-4-methoxybenzoic acid).

The second half of the ferutin molecule is the alcohol that we have called ferutinol. This has the composition  $C_{12}H_{20}O_2$ , mp 82-83°C (from water),  $\left[\alpha \right]_D^{25} + 38.3^\circ$  (c 1.02;  $CH_3OH$ ). Its IR spectrum has absorption bands at 3300-3500 cm<sup>-1</sup> (hydroxy groups) and 2880-2960 cm<sup>-1</sup> (C-methyl groups). Both oxygen atoms are present in hydroxy groups. Thus, ferutinol is a diol.

The NMR spectrum of ferutinol had a multiplet at 5.32 ppm (1H) due to an olefinic proton, a multiplet at 2.85 ppm (2H) caused by the protons of two hydroxy groups (which disappeared on deuteration), and a multiplet at 3.79 ppm (1H), corresponding to a proton geminal to a hydroxy group. The following signals were present in the strong-field region: singlet at 1.76 ppm (3H) of the protons of a methyl group on a double bond, singlet at 0.94 ppm from a tertiary methyl group, and two doublets at 0.91 and 0.83 ppm, J = 4 Hz (3H each) from two secondary methyl groups. In addition, the spectrum showed multiplets in the 1.5-2 ppm region with a total intensity of 4 H.

The mass spectrum of ferutinol had peaks with m/e 196 (M)<sup>+</sup>, 195 (M-1)<sup>+</sup>, 159 (M-1-2H<sub>2</sub>O)<sup>+</sup>, 151 (M-1-44)<sup>+</sup>, 135, 133. The peaks with m/e 177 and 159 confirm the presence of two hydroxy groups in the substance, and the peak with m/e 151 apparently corresponds to the fragment after the ejection of an isopropyl group. The presence of the latter in the molecule of the alcohol is in harmony with the NMR data.

The acetylation of ferutinol with acetic anhydride in pyridine gave a monoacetate  $C_{14}H_{22}O_3$  (M<sup>+</sup> 238) with mp 74-75°C (from ether), in the IR spectrum of which there were absorption bands at 3450 cm<sup>-1</sup> (hydroxy group) and 1735 and 1245 cm<sup>-1</sup> (ester carbonyl group). The absorption in the 3450 cm<sup>-1</sup> region shows that the product obtained is a monoacetate (under these conditions, a tertiary hydroxyl is not acetylated). The NMR spectrum of the monoacetate showed a three-proton singlet at 1.9 ppm (acetyl methyl group), a broadened one-proton signal of a tertiary hydroxyl at 2.25 ppm, and a one-proton multiplet at 4.80 ppm due to the geminal proton in an ester grouping. The results of a comparison of the NMR spectra of ferutin and the monoacetate showed that they are identical in the 0-5.5 ppm region and that the ferutinol is bound to the isovanillic acid through the secondary hydroxy group.

In view of the composition  $C_{12}H_{18}(OH)_2$  and the presence of only one double bond in the molecule, ferutinol must have a bicyclic structure.

The isopropyl group forming part of the side chain is the first isoprenoid unit in the ferutinol skeleton, and the second forms part of the ring; by the isoprene rule, the latter is probably attached to the first in the "head to tail" manner. The third residue, in the form of two carbon atoms cannot be between them and is connected with the second isopentenyl residue, i.e., with the ring.

In view of these considerations, and also the chemical and spectral observations, the alternative structures (I) and (II) may be suggested as the most probable for ferutinol.



Structure (I) is excluded on the basis of the stability of the diol to periodic acid oxidation, and therefore structure (II) is proposed for ferutinol. In that case, ferutin will have the following structure:



### EXPERIMENTAL

The IR spectra (tablets in KBr) were taken on a UR-10 spectrophotometer, the UV spectra (ethanolic solution) on a Hitachi instrument, the NMR spectra ( $CDCl_3$  and  $CCl_4$ ) on a JNM-4H-100/100 MHz spectrophotometer (internal standard HMDS), and the mass spectra on an MKh-1303 mass spectrometer fitted with a system for the direct introduction of substance into the ion source at 40 eV and 120°C. The purity of the substances was checked by chromatography on Silufol plates in the hexane-benzene-methanol (5:4:1) system. The elementary analyses corresponded to the calculated figures.

<u>Hydrolysis of Ferutin.</u> Production of ferutinol. A mixture of 0.2 g of ferutin and 20 ml of 5% aqueous caustic potash solution was heated in the water bath for 4 h. After cooling, the mixture was diluted with water, acidified with 10% sulfuric acid, and extracted with ether. The substances of acid nature were extracted from the ethereal solution with a 1% solution of caustic potash (solution A). The mother solution was washed to neutrality with water, dried over sodium sulfate, and distilled. After recrystallization from water, crystals were obtained with the composition  $C_{12}H_{20}O_2$ , mp 82-83°C,  $[\alpha]_D^{25} + 38.2^\circ$  (c 1.02; CH<sub>3</sub>OH),  $R_f$  0.55 (chromogenic agent 1% aqueous solution of KMnO<sub>4</sub>), M<sup>+</sup> 196.

Isolation of 3-hydroxy-4-methoxybenzoic acid. Solution A was acidified with 10% sulfuric acid and treated with ether. The residue after the solvent had been eliminated was crystallized from methanol. Crystals with the composition  $C_8H_8O_4$  deposited, mp 250°C,  $R_f$  0.00, M<sup>+</sup> 168. UV spectrum: 260 nm (log  $\epsilon$  2.90). Literature data for isovanillic acid - mp 250°C.

<u>3-Acetoxy-4-methoxybenzoic Acid.</u> A solution of 0.08 g of the acid in 4 ml of pyridine was treated with 3 ml of acetic anhydride. The mixture was left for a day at room temperature, and then the solvent was distilled off and the residue was extracted with ether. This gave 0.06 g of the acetate of the hydroxy acid with mp 214-215°C (from methanol),  $R_f$  0.00. Literature data for the acetate – mp 215°C.

<u>Ferutinol Acetate</u>. The substance (0.1 g) was acetylated as described above. This gave 0.09 g of ferutinol acetate with mp 74-75°C (from ether),  $R_f$  0.9 (chromogenic agent a 1% aqueous solution of KMnO<sub>4</sub>), M<sup>+</sup> 238.

Dihydroferutin. The substance (0.1 g) was hydrogenated in 15 ml of acetic acid in the presence of 0.05 g of PtO<sub>2</sub>. The amount of hydrogen absorbed was 6.3 ml, which corresponds to one double bond. The reaction product was extracted in the usual way and formed a colorless amorphous substance with  $R_f$  0.51, M<sup>+</sup> 348.

Acetylferutin. The substance (0.12 g) was acetylated with acetic anhydride in pyridine. On heating on the water bath for 4 h, 0.1 g of acetylferutin was isolated in the form of a colorless amorphous substance with  $R_f 0.7$ , M<sup>+</sup> 388.

<u>Diacetylferutin</u>. A solution of 0.1 g of the substance in 15 ml of acetic anhydride was treated with 0.5 g of freshly fused sodium acetate, and the mixture was heated at the boiling point for 6 h. The reaction product was isolated in the usual way. Yield 0.085 g – an amorphous substance with  $R_f$  0.82, M<sup>+</sup> 430.

### SUMMARY

The structure of a new diol – ferutin,  $C_{20}H_{26}O_5$  – with mp 130-131°C,  $[\alpha]_D^{25}$  + 101.8° (methanol), found in some representatives of the genus Ferula, has been established.

A study of the products of hydrolysis, hydrogenation, and acetylation, NMR, IR, and UV spectroscopy, and mass spectrometry have shown that ferutin is probably an ester of 3-hydroxy-4-methoxybenzoic acid and 1-(5-hydroxy-1,4-dimethylbicyclo[3,1,0]hex-3-en-2-yl)-2-methylpropanol.

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