COUMARINS AND ESTERS OF *Ferula microcarpa*

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Continuing a study of the coumarins and esters of plants of the genus *Ferula*, we have investigated the neutral and phenolic components of the roots of *Ferula microcarpa* Eug. Korov. collected in the Kyzart pass.

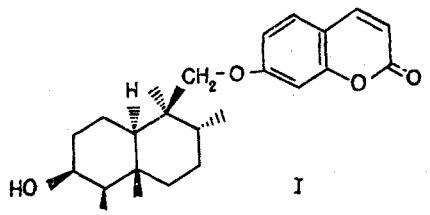
By chromatographing the neutral fraction of a methanolic extract on a column of silica gel, in addition to the kamolol [1] and feropolol [2], known previously, we have isolated a new terpenoid coumarin, $C_{24}H_{32}O_4$, M^+ 384, which we have called fecarpin (I).

The UV spectrum of (I) [λ_{max} 220, 243, 290, 327 nm (log ε 4.14, 3.58, 3.78, 3.98, resepctively)] is characteristic for umbelliferone derivatives. The IR spectrum shows absorption bands at 1615, 1570, and 1514 cm^{-1} (aromatic nucleus), 1715 cm^{-1} (C=O of an α-pyrone), and 3530 cm^{-1} (OH), confirming that fecarpin is a coumarin derivative. The hydroxy group in fecarpin is secondary — under mild conditions it is readily acetylated. A comparison of the PMR spectra of fecarpin and kamolol showed that they differ in their CS values and in the multiplicity of the signal of the hemihydroxylic proton (m, 3.58 ppm, $\Sigma_{1/2}$ = 9 Hz) and also by a slight change in the CSs of the signals of the methyl groups. These facts indicate that fecarpin is a stereoisomer of kamolol in relation to the orientation of the hydroxy group.

In fact, when (I) was oxidized with chromium trioxide in acetone, a compound with the composition $C_{24}H_{30}O_4$, M^+ 282, mp 190-192°C, was obtained which was identical according to its IR and PMR spectra with kamolone [1, 3].

Thus, the hydroxy group in fecarpin has the axial orientation and the configurations of the other asymmetric centers are the same as in kamolone [4].

On the basis of the facts given it may be concluded that fecarpin has the following structure and configuration:



I

By chromatographing the phenolic fraction of the extract on a column of silica gel, we isolated two esters, which we have called microferin, $C_{22}H_{28}O_3$ (II), M^+ 340, and microferinin, $C_{23}H_{30}O_4$ (III), M^+ 370. Both compounds are readily soluble in alkalis, benzene, chloroform, ether, and ethanol, sparingly soluble in hexane, and insoluble in water.

The UV spectrum of (II) shows a maximum at 260 nm (log ε 4.22), and the UV spectrum of (III) has maxima at 266 nm (log ε 4.02) and 296 nm (log ε 3.23) due to the presence of the p-hydroxybenzoyl and 3,4-dihydroxybenzoyl chromophores, respectively. In the presence of alkali, the long-wave maxima undergo bathochromic shifts by 44 and 54 nm, respectively, which shows the phenolic nature of both compounds.

The IR spectra of microferin and microferinin contain, in addition to the absorption bands of an aromatic nucleus and of hydroxy groups, the carbonyl bands of esters of phenolcarboxylic acids (1670 and 1690 cm^{-1}, respectively).

On alkaline hydrolysis, (II) and (III) formed the same sesquiterpene alcohol — microferol, $C_{15}H_{24}O$ (IV), M^+ 220 — and acids: in the case of microferin, p-hydroxybenzoic acid, and in the case of microferinin vanillic acid.

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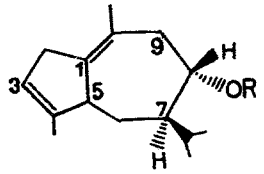
The PMR spectrum of the alcohol (IV) showed the following signals: broadened singlet at 5.29 ppm (1H, $\sum_{1/2}$ = 6.7 Hz, $-\underline{C}H=\underline{C}-$), singlet at 1.59 ppm (6H, $2C=\underline{C}-\underline{C}H_3$), two doublets at 0.83 and 0.96 ppm (3H each, J = 7 Hz, $-CH(\underline{C}H_3)_2$), and a septet at 3.79 ppm (1H, ^3J = 5.5, 9.0, 10.5 Hz, $\underline{H}-\underline{C}-OH$). When (IV) was acetylated with acetic anhydride in pyridine, a monoacetate $C_{17}H_{26}O_2$ was obtained in the PMR spectrum of which the signal of the hemihydroxy group had undergone a paramagnetic shift by 1.08 ppm.

On the basis of the composition and chemical and spectral characteristics, we propose for the alcohol the bicyclic guaianane structure (IV), which was confirmed by the formation of guaiazulene from (II) and (III) on dehydrogenation with selenium at 200-230°C.

The position of the hydroxy group at C_8 in microferol is unambiguously determined by the nature of the splitting of the signal of the hemihydroxylic proton.

Thus, microferin and microferinin are esters of the guaiane alcohol microferol (IV) with p-hydroxybenzoic and vanillic acids, respectively.

The orientations of the ester group and of the isopropyl radical in (II) and (III) follow from the SSCC of the signal of the hemiacyl proton. In the spectra of (II) and (III) the latter appears in the form of a septet at 5.16 ppm with ^3J = 5.5, 9.0, and 10.5 Hz, which shows the equatorial orientations [5] of the ester and isopropyl groups in microferin and microferinin. It must be mentioned that the microferinin that we isolated proved to be identical with a compound obtained previously by the cyclization of chimganidin in an acid medium [6]. On the basis of the fact given, for compounds (I) and (II) we propose the following structures and configuration:



II. R=OC—C_6H_4—OH
III. R=OC—C_6H_3(OH)(OCH_3)
IV. R=H

The ease of cyclization of chimganidin to microferinin in 10% ethanolic acid solution may give rise to doubt concerning the native nature of (II) and (III), but these substances are present in the fresh raw material. Furthermore, it has been checked experimentally that their formation under the conditions of isolation is excluded because we are dealing with dilute aqueous solutions of acids.

Ferula microcarpa (section Xeronarthex) is the second representative of plants of the genus *Ferula* in which esters have been found in addition to coumarin derivatives. These groups of natural compounds were first isolated from *F. korshinskyi* (section Macrochriza) [7, 8].

EXPERIMENTAL

The UV spectra were taken on an Hitachi instrument in ethanolic solutions, the IR spectra on a UR-20 spectrophotometer (tablets with KBr, in films), the mass spectra on an MKh-1303 mass spectrometer, and the PMR spectrum on a JNM-4H 100/100 MHz instrument (in $CDCl_3$). The chemical shifts are given in the δ scale from the signal of HMDS taken as zero.

The purity of the substances and the course of the reactions were checked by the TLC method on Silufol in chloroform—ethyl acetate (4:1) (for coumarins) and (25:1) (for esters).

The coumarins were detected in UV light and the esters were revealed with a 1% solution of vanillin in concentrated sulfuric acid and a 3% solution of potassium permanganate.

Isolation of the Coumarins. The dried and comminuted roots were extracted with ethanol (3 × 5 liters). The extract was concentrated, diluted with water (1:2), and extracted with ether (4 × 0.25 liter). The ethereal extract was treated with 1% caustic potash solution and was then washed with water and dried, and the solvent was distilled off. This gave 60 g of a resinous residue, 50 g of which was deposited on a column of KSK silica gel (3.5 × 110 cm). The coumarins were eluted (2:1) [sic], 100-ml fractions being collected.

When the eluate was concentrated, fractions 12-15 yielded crystals of fecarpin (0.16 g), mp 166-168°C (hexane—chloroform), $[\alpha]_D^{20}$ −20° (c 1.0; chloroform), R_f 0.46; fractions 21-29 gave kamolol (0.62 g), mp 140-142°C, R_f 0.32; and the subsequent fractions gave feropolol (0.27 g), mp 94-96°C, $[\alpha]_D^{21}$ +38° (c 1.1; chloroform), R_f 0.14.

Acetylation of Fecarpin. A solution of 35 mg of fecarpin in 1 ml of pyridine was treated with 1 ml of acetic anhydride. The mixture was heated in the water bath for 1.5 h. The acetyl derivative was isolated in the usual way ($C_{26}H_{34}O_5$, M^+ 426, R_f 0.64).

Oxidation of Fecarpin. With stirring, a solution of 65 mg of chromium trioxide in 1 ml of water was added dropwise to a solution of 55 mg of fecarpin in 10 ml of acetone. After 1 h, the mixture was diluted with ice water and the precipitate that deposited was filtered off, washed with water, and recrystallized from acetone. This gave 42 mg of a substance $C_{24}H_{30}O_4$, M^+ 382, mp 190-192°C, $[\alpha]_D^{20}$ +50° (c 1.0; chloroform).

Isolation of the Esters. The alkaline solution was neutralized with 5% sulfuric acid and was treated with ether. The ethereal extract was washed with water and dried, and the solvent was driven off. This gave 31 g of a phenolic fraction, 9 g of which was chromatographed on a column of silica gel (1.7 × 122 cm). The substances were eluted with the benzene—ethyl acetate (9:1) system, 50-ml fractions being collected. Fractions 11-14, when rechromatographed on silica gel (0.8 × 12 cm, benzene, fractions 4-12, 10 ml each), yielded 0.25 g of crystalline substance (III) with the composition $C_{23}H_{30}O_4$ (M^+ 370), mp 152-154°C (hexane), $[\alpha]_D^{24}$ +89.3° (c 0.9; $CHCl_3$), R_f 0.47.

On rechromatography (under the same conditions), fractions 20-24 yielded 0.24 g of compound (II) with the composition $C_{22}H_{28}O_3$ (M^+ 340), mp 144-145°C (hexane), $[\alpha]_D^{23}$ +122.9° (c 0.8; $CHCl_3$), R_f 0.25.

Hydrolysis of Microferin. A solution of 110 mg of (II) in 10 ml of 10% aqueous KOH solution was heated in the water bath for 4 h. The reaction mixture was diluted with water and treated with ether. The ethereal extract was washed with water and was dried with sodium sulfate, and the solvent was distilled off. This gave 56 mg of an oil substance with the composition $C_{15}H_{24}O$ (IV), $[\alpha]_D^{25}$ +90° (c 1.0; $CHCl_3$), R_f 0.78.

The mother liquor was acidified with 5% sulfuric acid and treated with ether. Evaporation of the solvent yielded an acid with the composition $C_7H_6O_3$, mp 210-212°C, identical with p-hydrobenzoic acid.

Hydrolysis of Microferinin. The hydrolysis of 0.12 g of (III) was carried out by the method described above. This gave microferol (IV) and vanillic acid, $C_8H_8O_4$, mp 205-206°C.

Acetylation of Microferol. The alcohol (65 mg) was acetylated with acetic anhydride (1.5 ml) in pyridine (3 ml) for 30 min. The reaction product was isolated in the usual way. This gave the monoacetate in the form of an oil with the composition $C_{17}H_{26}O_2$, R_f 0.85.

Cyclization of Chimganidin. A mixture of 250 mg of chimganidin and 20 ml of 10% ethanolic sulfuric acid was left at room temperature for 20 min. After the end of the reaction, the mixture was diluted with water, and the reaction product was extracted with ether. This gave a crystalline substance with the composition $C_{23}H_{30}O_4$, mp 152-153°C (hexane), identical with microferinin.

Dehydrogenation of Microferin. A mixture of 130 mg of (II) and 400 mg of selenium was heated at 200-230°C for 5 h. The dehydrogenation product was extracted with petroleum ether and passed through alumina. This gave a blue oil the picrate of which had mp 120-121°C.

The dehydrogenation of microferinin was performed similarly. The picrate of the reaction product melted at 120-121°C.

SUMMARY

1. In addition to known compounds, a new terpenoid coumarin has been isolated from *Ferula microcarpa* — fecarpin, which is the stereoisomer of kamolol in relation to the orientation of the hydroxy group.

2. Microferin and microferinin — esters of a new guaiane alcohol microferol with p-hydroxybenzoic and vanillic acids, respectively — have been isolated.

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O-ACYLATED FLAVONOID GLYCOSIDE OF THE NEEDLES OF *Picea obovata*

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The needles of plants of the family *Pinaceae* have recently attracted attention as a source of O-acylated flavonoid compounds [1-6]. It is interesting to note that in both the genera *Pinus* and *Picea* the acylating agents are phenolic acids that are typical for coniferous plants, particularly p-coumaric acid [2-5]. At the same time, a little-known form of acylation has been observed: simultaneous acylation by two p-coumaric acid residues [3, 4].

The present work was devoted to determining the structure of a new compound (I) which we have isolated from the needles of *Picea obovata* Ledeb. (Siberian spruce) and is of interest as an example of a flavonol glycoside acylated simultaneously by residues of two phenolic acids characteristic for spruce needles — p-coumaric and ferulic acids [7].

From the results of hydrolytic cleavage and its PMR spectrum (Fig. 1), compound (I) includes residues of kaempferol, glucose, and p-coumaric and ferulic acids. The substance is very stable under conditions of severe acid hydrolysis (25% HCl, 100°C), which does not go to completion even after boiling for 48 h. Kaempferol was detected among the hydrolysis products but none of its acyl derivatives.

From its IR spectrum the substance can be characterized as an ester (ν_{CO} 1690, 1710 cm^{-1}). The UV spectra measured by a standard method contain a very broad band with a maximum at 320 nm, which does not permit the free OH groups in kaempferol to be differentiated; a 5-OH group in (I) was revealed by its PMR spectrum.

The glycosidation of kaempferol at the 3-OH group was established on the basis of the UV spectra of the deacylated product (II), which was identical with astragalin (kaempferol 3-O-β-D-glucopyranoside). The mild saponification of compound (I) led to the appearance even in the first 10-15 min of two monoacylated compounds (III) and (IV), the latter practically disappearing after 20-30 min. From the products of alkaline hydrolysis we isolated astragalin (II), astragalin ferulate (III), and p-coumaric and ferulic acids (Scheme 1).

The β-orientation of the glycosidic bond of the D-glucopyranose in compounds (I-III) is shown by the optical rotation of these glycosides and by the diaxial coupling constant of the anomeric proton in their PMR spectra.

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