ESTERS OF *Ferula rubroarenose*

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UDC 547.992:547.37

Continuing a study of the esters of plants of the genus *Ferula*, we have investigated the chemical composition of the roots of *Ferula rubroarenosa* Eug. Kor. collected in Kirghizia in the fruit-bearing phase.

By chromatographing the phenolic fraction of an ethanolic extract of the roots we isolated nine esters, six of them being identified on the basis of their physicochemical constants, mixed melting points of the product of alkaline cleavage, and spectral characteristics (IR, PMR) as the previously known *l*-chimgin [1], ferolin [2], chimganidin [3], federin [4], feringin [5], and teferin [6].

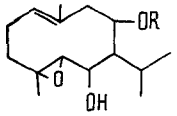
Three esters with the compositions $C_{18}H_{24}O_4$ (M^+ 304), $C_{25}H_{32}O_6$ (M^+ 404), and $C_{22}H_{30}O_5$ (M^+ 374) were new, and we have called them rubaferin (I), rubaferinin (II), and rubaferidin (III), respectively. The substances are readily soluble in ethanol and ether, and sparingly soluble in hexane. The UV spectra of (I) and (II) each have the maxima characteristic for a 3,4-dihydroxybenzoyl group [(I) — λ_{max} 263, 298 (log ε 3.94, 3.68); (II) — λ_{max} 265, 295 (log ε 4.01, 3.79)], and the UV spectrum of (III) is characterized by the presence of a p-hydroxybenzoyl chromophore [λ_{max} 261 (log ε 4.19)]. In the presence of alkali, the short-wave maxima in the spectra of (I)-(III) undergo a bathochromic shift with the simultaneous increase in intensity, which indicates the presence of free phenolic hydroxy groups.

Each of the IR spectra (I-III) has the absorption bands of an aromatic nucleus (1515-1620 cm^{-1}), of the carbonyl group of an ester of an unsaturated acid (1675-1710 cm^{-1}), and of a hydroxy group (3200-3400 cm^{-1}).

On alkaline hydrolysis rubaferin formed *l*-borneol, shown to be identical with an authentic sample on the basis of a mixed melting point and the value and sign of the specific rotation and its IR spectrum, and also isovanillic acid (mp 250-251°C). Thus, rubaferin is the previously unknown *l*-borneol ester of isovanillic acid.

In the PMR spectrum of rubaferinin (II) in the strong-field region there are signals of the methyls of an isopropyl group at 0.92 and 1.15 ppm (d, 3 H each, J = 6 Hz), of methyl groups in an epoxide ring at 1.15 ppm (s, 3 H) and on a double bond at 1.77 ppm (s, 3 H), and also the signals of an epoxy proton at 2.8 ppm (d, 1 H, J = 7 Hz) interacting with a gem-hydroxylic proton at 3.84 ppm (d, 1 H, J = 7 Hz). At 5.3 ppm appear the signals of olefinic and hemiacyl protons (m, 2 H). At 3.88 ppm (OCH_3) and in the weak-field region there are the signals of the protons of a vanillic acid residue. The PMR spectrum of rubaferidin differs from the spectrum of (II) only by the signals of the acid residue which in this case is that of p-hydroxybenzoic acid.

The hydrolysis of (II) and (III) gave the same sesquiterpene alcohol with the composition $C_{15}H_{26}O_3$, mp 87-88°C, identical with ugamdiol (shiromodiol) [7, 8]. Vanillic and p-hydroxybenzoic acids, respectively, were isolated from the acid fractions of the hydrolysis products. The chemical shift and the multiplicity of the signal of the hemiacyl proton in the PMR spectrum of (II) and in that of (III) show that the acid residues in rubaferinin and rubaferidin are attached to the hydroxyl remote from the epoxy group, i.e., at C_8.



II. R = C_6H_3–(OH)(OCH_3)–CO–

III. R = C_6H_4(OH)–CO–

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 712-715, November-December, 1978. Original article submitted July 4, 1978.

 0009-3130/78/1406-0606$07.50

On the basis of the facts given we have concluded that rubaferinin and rubaferidin have the structure of 8-vanilloyl- and 8-p-hydroxybenzoylugamdiol, respectively. It is interesting to note that *Ferula rubroarenosa* is still the only representative of plants of the genus *Ferula* containing simultaneously esters of four alcohols with different terpene and sesquiterpene skeletons: borneol (camphane), angrendiol, ugamdiol (germacrane), and ferutinol (carotane).

EXPERIMENTAL

The conditions for recording the spectra have been given previously [9].

Isolation and Separation of the Esters. An ethanolic extract from the roots (1.4 kg), after concentration, was diluted with water and treated with ether. The ethereal extract was treated with a 5% solution of sodium carbonate and with a 1% solution of caustic potash. The alkaline solution was acidified with sulfuric acid and the phenolic components were extracted with ether. The solvent was distilled off. This gave 140 g of the phenolic fraction. Of this, 15 g was deposited on a column of silica gel (100 × 3.5 cm) and the substances were eluted with chloroform, 50-ml fractions being collected. The fractions having the same composition in the chloroform–ethyl acetate (25:1) system were combined (11-16, 17-24, 28-31, 35-37, 38-49, and 72-77) and were rechromatographed or crystallized.

Isolation of Rubaferin, Chimgin, and Federin. After elimination of the solvent and recrystallization from hexane, fractions 11-16 yielded 1.6 g of rubaferin, $C_{18}H_{24}O_4$ (M^+ 304), mp 106-107°C, $[\alpha]_D$ −45.08° (c 0.66; chloroform).

Fractions 28-31 yielded 0.39 g of chimgin, $C_{17}H_{22}O_3$, mp 158-160°C (hexane), $[\alpha]_D$ −40.76° (c, 0.66; ethanol), and fractions 35-37 gave 0.11 g of federin, $C_{24}H_{32}O_5$, mp 178-179°C [hexane–ether (5:1)], $[\alpha]_D$ −80.0° (c 1.0; ethanol).

Isolation of Teferin and Chimganidin. Fractions 17-24 were rechromatographed on a column of silica gel (65 × 1.3 cm). Fractions 18-24 yielded 0.13 g of teferin, $C_{23}H_{32}O_5$, mp 78-80°C, $[\alpha]_D$ +82.4° (c 0.9, chloroform), and fractions 36-51 gave 0.27 g of chinganidin, $C_{23}H_{32}O_5$, mp 140-141°C [hexane–ether (7:1)], $[\alpha]_D$ −80°C (c 1.0; chloroform).

Isolation of Ferolin, Feringin, and Rubaferinin. Fractions 38-49 were chromatographed on a column of silica gel (110 × 1.5 cm). Feringin (0.82 g) crystallized out from fractions 9-21, $C_{22}H_{30}O_4$, mp 82-84°C [hexane–ether (7:1)], $[\alpha]_D$ +70.0° (c 1.0; chloroform), and ferolin (0.81 g) from fractions 25-37, $C_{22}H_{30}O_4$, mp 105-106°C [hexane–ether (9:1)], $[\alpha]_D$ −36° (c 1.0; chloroform).

Isolation of Rubaferidin. The residue obtained after the distillation of the solvent from fractions 72-77 was rechromatographed on a column of silica gel (60 × 1 cm); fractions 15-41 were combined and the solvent was distilled off. This gave 0.3 g of rubaferidin, $C_{22}H_{30}O_5$ (M^+ 374), mp 162-164°C [hexane–ether (9:1)], $[\alpha]_D$ −40° (c 1.1; chloroform).

Hydrolysis of Rubaferin [1]. With heating, 120 mg of the substance was hydrolyzed with 80 ml of a 5% aqueous solution of caustic potash for 40 min. The reaction mixture was diluted with water and treated with ether. After elimination of the solvent, borneol $C_{10}H_{18}O$ was obtained, mp 201-202°C, $[\alpha]_D$ −40° (c 0.5; chloroform). The aqueous solution after the ether treatment was acidified with 5% sulfuric acid. On standing, the solution deposited acicular crystals of isovanillic acid, $C_8H_8O_4$, mp 250-251°C.

Hydrolysis of Rubaferinin (II) and Rubaferidin (III). Hydrolysis was performed by the method described above. The neutral fractions of the hydrolyzates of (II) and (III) yielded ugamdiol ($C_{15}H_{26}O_3$, mp 87-88°C), and the acid fractions yielded vanillic acid ($C_8H_8O_4$, mp 205-206°C) and p-hydroxybenzoic acid ($C_7H_6O_3$, mp 209-210°C), respectively.

SUMMARY

Three new esters, in addition to six known esters, have been isolated from the roots of *Ferula rubroarenosa* Eug. Kor. On the basis of chemical and spectral characteristics rubaferin, rubaferinin, and rubaferidin have been assigned the structures of isovanilloylborneol, and 8-vanilloyl- and 8-p-hydroxybenzoylugamdiol, respectively.

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A GAS-CHROMATOGRAPHIC METHOD OF DETERMINING LEDOL IN THE ESSENTIAL OIL, LEAVES, AND HERBAGE OF *Ledum palustre*

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UDC 543.8.51:665.526.15.002.3

The herb *Ledum palustre* L. (crystal tea ledum; march tea) has long been used in medicine. It is considered that the therapeutic action of this plant is connected with the terpenoid compounds present in the essential oil [1]. The amount of the essential oil and its composition depend strongly on the vegetation phase, the site and conditions of growth, etc. In view of this, the dependence of the amount and the physicochemical properties of the essential oil have been studied as functions of the times of collection, the vegetation phase, the growth site, and the plant organ, and the composition and structure of individual components of the oil have also been investigated [2-7].

It has been found that the predominating amount of essential oil is concentrated in the leaves, and the amount of essential oil in the other organs of the plant is insignificant. The greatest amount of essential oil is found in the leaves in the phase of the ripening of the fruit [2-7]. This is the time (August-September) at which it is recommended to collect the raw material [8].

The main components of the essential oil of *L. palustre*, which has a complex chemical composition, are palustrol, ledol, and (in young leaves) myrcene, and there are smaller amounts of p-cymene, geranyl acetate, hydrocarbons, etc. In the essential obtained from fresh leaves of *L. palustre* collected in Finland, 60 components (60 peaks on a chromatogram) have been detected, 18 of which predominate, the others being present in very small amounts (less than 1%) [9].

The amount of the main components — palustrol and ledol — falls in the period of the shedding of the old and the putting forth of the new leaves, and then it rises again, reaching its maximum in September. There is a hypothesis that one of the pharmacological effects of the herb *L. palustre* is due to the ledol [10], a bicyclic sesquiterpene alcohol with the composition $C_{15}H_{25}O$, mp 105-106.5°C.

We have developed a method for the quantitative determination of ledol in the essential oil of *L. palustre* which is based on the gas-chromatographic separation of the components of the essential oil under programmed temperature conditions in the presence of a known amount of methyl myristate (internal standard). Fairly complete separation (no less than 32 components being detected on the chromatogram) is achieved. The methyl myristate peak does not mask any of the peaks of the essential oil, as has been checked on a large number of samples (more than 100) of the essential oil obtained from the leaves of plants of various vegetation phases and from different growth sites.

On chromatograms of samples of essential oil with a high ledol content the largest peak is that of palustrol, the ledol peak being considerably smaller, and the peaks of the other

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 715-718, November-December, 1978. Original article submitted July 25, 1978.

 0009-3130/78/1406-0608$07.50