DITERPENOIDS OF *Teucrium polium*. THE STRUCTURE OF TEUCRIN P_1

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Several years ago, Brieskorn [1] isolated from the plant *Teucrium polium* L. picropolin — a diterpene furolactone of the clerodane type — and studied its structure. We have investigated certain extracts of a plant of the same species collected in the Rybnitsa region of the Moldavian SSR in order to study the substances accompanying picropolin. However, picropolin itself was not detected in these extracts. Instead of it we isolated and characterized two new diterpenoids [2] consisting of a furoacetal and a furolactone with a rearranged labdane carbon skeleton. In view of the structural linkage of the substances with the teucrins isolated previously from *Teucrium chamaedrys* L. [3], we proposed for them the name teucrins P_1 and P_2 (in order of their isolation from the extract).

Teucrin P_1, $C_{20}H_{24}O_5$, is the main component of the combined diterpenoid. The present paper gives information on the determination of its chemical structure.

According to its IR spectra the molecule of this compound contains a keto group (1710 cm^{-1}) and a furan ring (1590, 1505, and 880 cm^{-1}) but no hydroxy groups. According to elementary analysis, mass spectrometry, and the results of hydrogenation, teucrin P_1 does not contain olefinic bonds other than the two in the furan nucleus. Dehydrogenation with selenium led to the formation of naphthalene derivatives, especially 1,2,5-trimethylnaphthalene which shows the bicyclic labdane or clerodane carbon structure of this diterpenoid. However, in the IR and PMR spectra of teucrin and also in the spectra of its reduction products (II-VI) there are no signals of the gem-dimethyl groups characteristic of labdane compounds (doublet at 1375 cm^{-1}; signals of the protons of tertiary methyls in the strong-field region). This fact, and also those presented below, have enabled us to assign the diterpenoid isolated to compounds with a rearranged labdane skeleton, i.e., to the clerodane type of compound, as in the case of picropolin [1].

It also follows from the results given that three out of the five oxygen atoms of the teucrin P_1 molecule (apart from the furan and carbonyl oxygen atoms) must be present in the rings. On the basis of the chemical transformations shown below and an analysis of the spectral characteristics of the natural substance and its derivatives, it has been concluded that these oxygen atoms belong to oxide and acetyl rings.

Thus, the mass spectrum of teucrin P_1 has strong peaks with m/e 81 (40%) and 94 (100%), which are also characteristic for the spectra of picropolin and of all known teucrins [1, 3] and are assigned, respectively, to the ions formed as the result of the double fragmentation of the side chain at the C_{12}—O, C_{11}—C_{12}, and C_{12}—O, C_9—C_{11} bonds. It follows from this that the structure of the hydrocarbon side chain in all the diterpenoids mentioned is the same, and in this case the carbonyl group of teucrin P_1 can be present only in carbon rings.

By reducing teucrin P_1 with lithium tetrahydroaluminate, we obtained a diol (II), the PMR spectrum of which contained, in addition to the signal of the secondary methyl group of the starting material, the signal of a tertiary methyl group geminal to a hydroxyl (1.30 ppm). This shows the reduction of the terminal epoxide group which can occupy positions C_4-C_{19} or C_8-C_{20} in the clerodane skeleton. The diol (II) is not oxidized by sodium periodate, from which it follows that its hydroxy groups are not vicinal. Acetylation of the diol (II) with acetic anhydride in pyridine gave the monoacetate (III); oxidation with chromium trioxide gave the ketone (IV); and hydrogenation gave the tetrahydro derivative (V), which, af-

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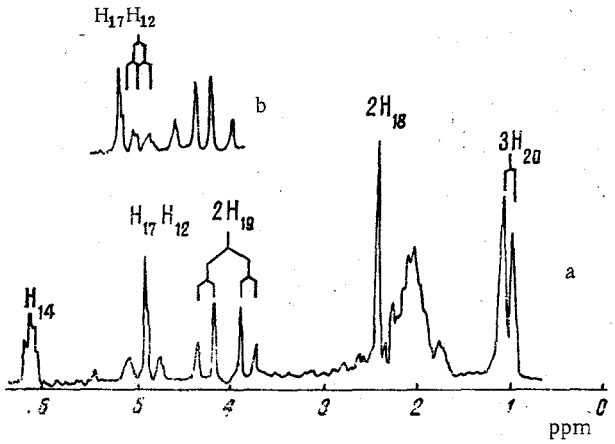
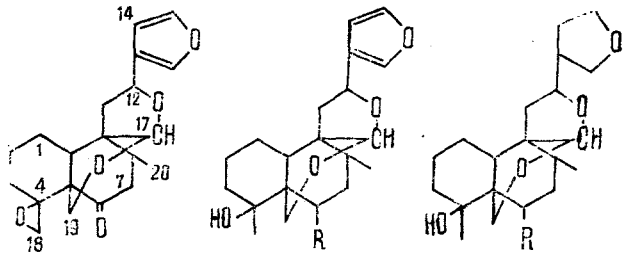
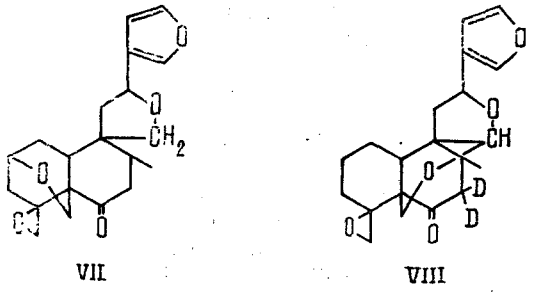


Fig. 1. PMR spectrum of teucrin P_1 in chloroform (a) and in pyridine (b).

ter oxidation with chromium trioxide, was converted into the saturated ketone (VI). The latter was also obtained by the direct hydrogenation of teucrin P_1 in ethanol or in acetic acid in the presence of palladium or platinum.



I II, III, IV V, VI



VII VIII

II, V. R=OH III. R=OAc IV, VI. R= =O

To determine the position of the carbonyl group we used a mass-spectrometric study of derivatives labelled with deuterium. After the deuteration of teucrin P_1 in an alkaline medium with the aid of D_2O we obtained a product (VIII) containing two deuterium atoms, as was confirmed by its mass spectrum. As it is not difficult to see when the results of the periodate oxidation of the diol (II) are taken into account, the replacement of two hydrogen atoms by deuterium is possible only if the keto group is located at C_6. Then the epoxide ring must occupy the C_4-C_{19} position since after the dehydration of the alcohol (VI) with thionyl chloride in pyridine no α,β-unsaturated ketone is formed as would be expected if the epoxide group were located at C_8-C_{20}.

All these facts and also a projection of the molecule of the diterpenoid in Stuart—Briegleb models have permitted the assumption for teucrin P_1 of only two types of structures with a clerodane skeleton: the acetal type (I) and an oxide type including one of the atoms C_1, C_2, and C_3 in the heterocycle as, for example, (VII). The choice in favor of structure

(I) was made on the basis of a study of the PMR spectra of teucrin P_1 of compounds (II) and (IV).

In the case of a structure of type (VII), in the low-field region of the spectrum of teucrin P_1 there should be the signals of two isolated groups in the form of AB systems ($2H_{17}$, $2H_{19}$) and one triplet of the H_{12} proton. In actual fact, in its spectrum one two-proton AB signal centered at 4 ppm and two one-proton signals in the 4.9 ppm region due to H_{12} and H_{17} are found. The signals of the two latter protons are resolved better when the spectrum is taken in pyridine (Fig. 1). Consequently, the structure of teucrin P_1 corresponds to the structure given as (I), which contains an acetal ring and also explains the instability of the natural product and its derivatives (II), (IV), and (VI) in a 1 N solution of sulfuric acid on heating.

EXPERIMENTAL

The IR spectra were taken on a UR-20 spectrometer in chloroform and the PMR spectra on a RS-60 instrument in chloroform (internal standard — tetramethylsilane); the melting points of the substances were determined on a Kofler block. For thin-layer chromatography (TLC) we used type KSK silica gel in a fixed layer, and also prepared plates of the Silufol type. The analyses of all the compounds corresponded to the calculated figures.

Isolation of Teucrin P_1. The plant (2 kg) was extracted by the method of steeping in acetone. The infusion was evaporated in vacuum in a current of inert gas to 1 liter and was then diluted with water (1:1) and extracted with chloroform. The solvent was distilled off and the viscous light-colored residue (20 g) was chromatographed on type L 100/250 μ silica gel, a Czech product, with eluting mixtures of gradually increasing polarity, beginning with chloroform and ending with methanol. The first fraction contained the teucrin P_1 [2], which was purified by rechromatography and crystallization. Yield: 0.05% on the weight of the dry plant. $C_{20}H_{24}O_5$, (I) $[\alpha]_D$ 21.4° (c 4.66; chloroform), R_f 0.74 [benzene—acetone (2:1)]. PMR spectrum, ppm: 1.00 (doublet, 2 H, J = 6 Hz, methyl at C_8) 2.43 (2H, methylene protons at C_{18}), 3.80 and 4.25 (2H, AB, J = 10.5 Hz, methylene protons at C_{19}), 4.88 (triplet, 1H, J = 7 Hz, proton at C_{12}), 4.91 (singlet, 1H, proton at C_{17}, 6.15 (1H, β-H of a furan).

Dehydrogenation of Teucrin P_1. The substance (0.5 g) was first hydrogenated in the presence of Adams oxide in acetic acid. The total material isolated was heated in a tube at 300°C for 40 h. Then the reaction mixture was ground and extracted with ether. The total material isolated was chromatographed on a column of alumina (activity grade II). In a petroleum ether eluate, consisting of the hydrocarbon fraction, 1,2-dimethylnaphthalene and 1,2,5-trimethylnaphthalenes, as the main components of the mixture, were identified by the GLC method (15% of Reoplex-400 on Chromaton N-AW DMCS, temperature 180°C) in the presence of markers.

Reduction of Teucrin P_1. A mixture of 200 mg of the substance and 300 mg of lithium tetrahydroaluminate in 5 ml of dry tetrahydrofuran was boiled under reflux for 4 h. After the usual working up and chromatographic purification of the residue, 180 mg of a diol $C_{20}H_{28}O_5$ (II) with mp 162-164°C was isolated. IR spectrum, cm^{-1}: 3600, 3400 (OH), 1620, 1510, 880 (furan); there were no bands characteristic for C=O. PMR spectrum, ppm: 0.93 (3H, doublet, J = 6 Hz), 1.30 (3H, singlet), 3.5-4.3 (5H, multiplet) 4.88 (1H, singlet), 4.95 (1H, triplet, J = 7 Hz), 6.18 (1H).

Preparation of the Monoacetate (III). A solution of 250 mg of the diol (II) in 3 ml of pyridine was treated with 0.5 ml of acetic anhydride and the mixture was left overnight. After the usual working up and chromatographic purification on silica gel, 150 mg of the monoacetate (III) was obtained; it consisted of a noncrystallizing liquid that was converted into a dry foam on drying in a high vacuum. $C_{22}H_{30}O_6$ (III). IR spectrum, cm^{-1}: 3640, 1740, 1620, 1505, 1240, 880. PMR spectrum, ppm: 0.95 (3H, doublet, J = 6 Hz), 1.25 (3H, singlet), 2.0 (3H, singlet), 3.65 and 4.3 (2H, AB, J = 11.5 Hz), 4.88 (1H, singlet), 4.73-5.05 (2H, unresolved multiplet), 6.18 (1H).

Oxidation of the Diol (II). L. To 25 mg of the substance in a mixture of 0.6 ml of methanol and 0.2 ml of water was added 50 mg of sodium metaperiodate in aqueous methanol. The substance obtained after three days' standing was identical with the starting material.

II. To a complex of 50 mg of chromium trioxide in pyridine was added a solution of 20 mg of the substance in 0.5 ml of pyridine and the mixture was left at room temperature for

4 h. After the usual working up and chromatographic purification on silica gel with a mixture of benzene and 5% of acetone, a crystalline substance was eluted. $C_{20}H_{26}O_5$ (IV), mp 171-174°C. IR spectrum, cm^{-1}: 3480, 1690, 1500, 870.

Preparation of the Ketone (VI). I. A solution of 210 mg of the diol (II) in 5 ml of acetic acid was saturated with hydrogen in the presence of 30 mg of platinum oxide (according to Adams) with stirring for 90 min. The amount of hydrogen absorbed was 45.56 ml (under normal conditions). After chromatographic separation of the mixture obtained, the tetrahydro derivative $C_{20}H_{32}O_5$ (V) was isolated. IR spectrum, cm^{-1}: 3580, 3400; there were no bands characteristic of a furan nucleus.

II. The substance (V) (50 mg) was oxidized with chromium trioxide in pyridine by the method described above. After chromatographic purification 30 mg of a saturated ketone $C_{20}H_{30}O_5$ (VI) with mp 155-156°C was obtained. IR spectrum, cm^{-1}: 3620, 3480, 1700.

Dehydration of the Ketone (VI). With cooling to 0°C, 1 ml of thionyl chloride was added dropwise to a solution of 260 mg of the substance in 3 ml of dry pyridine. The mixture was left for 2 h, and then ice was added and the reaction products were extracted with chloroform. The extracts obtained were washed, dried, and distilled. The residue (220 mg) was chromatographed. IR spectrum, cm^{-1}: 3020, 1660 (double bonds), 1705 (C=O); no bands characteristic of hydroxy groups were present.

Hydrogenation of Teucrin P_1. In the presence of $Pd/BaSO_4$, 170 mg of the substance in 6 ml of concentrated acetic acid was saturated with hydrogen for 5 h, 2.68 equivalents of hydrogen being absorbed. Chromatography on SiO_2 yielded as main reaction products a saturated ketone $C_{20}H_{30}O_5$, mp 154-156°C. IR spectrum, cm^{-1}: 3620, 3480, 1700; there were no bands characteristic for a furan nucleus. The IR spectrum was identical with that of the ketone (VI). PMR spectrum, ppm: 0.98 (3H, doublet, J = 6 Hz), 1.22 (3H, singlet), 3.5-4.4 4.4 (three AB systems), 4.9 (1H, singlet). A mixture with the ketone (VI) gave no depression of the melting point.

Deuteration of Teucrin P_1. To 0.5 ml of absolute dioxane were added 0.1 ml of D_2O and 10 mg of metallic sodium, and then 15 mg of teucrin P_1 dissolved in 0.5 ml of absolute dioxane. The mixture was boiled under reflux with protection from atmospheric moisture for 15 min. Then it was distilled to dryness. The residue was treated with 0.5 ml of dioxane and 0.1 ml of deuterium oxide. It was again boiled and was distilled to dryness. The new residue was dissolved in dry chloroform and the solution was washed with 0.1 ml of D_2O. The chloroform layer was separated off and the product was isolated by the usual method. This gave a solid crystalline substance $C_{20}H_{22}D_2O_5$, mp 164-167°C, M^+ 346.

SUMMARY

The structure of the diterpenoid teucrin P_1, isolated from *Teucrium polium* L., has been established on the basis of chemical reactions and spectral analysis.

LITERATURE CITED

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