LITERATURE CITED

1. M. Shipchandler and T. O. Soine, J. Pharm. Sci., 57, 741 (1968).
2. P. L. Zakharov, V. S. Kabanov, A. L. Ban'kovskii, G. K. Nikonov, and N. E. Ermatov, Khim. Prirodn. Soedin., 398 (1971).
3. P. L. Zakharov, P. B. Terent'ev, G. K. Nikonov, and A. L. Ban'kovskii, Khim. Prirodn. Soedin., 431 (1972).
4. R. G. Cooks and D. H. Williams, Chem. Commun., 663 (1968).
5. M. Paal, Z. Naturforsch., 96, 418 (1954).
6. M. Shipchandler and T. O. Soine, J. Pharm. Sci., 57, 2062 (1968).
7. R. Dzhonston, Handbook on Mass Spectrometry for Organic Chemists [in Russian], Moscow (1975).

DITERPENE LACTONES OF Teucrium hyrcanicum

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UDC 547.913

Species of germander (Teucrium) have long been used in officinal folk medicine as antiflammatory, hypotensive, cholagogic, antihelminthic, etc., agents [1-6]. The species T. polium L. [7], T. Chamaedrys L. [8, 9], and T. viscidum Bl. [10] have been studied chemically to the greatest extent, and from them a number of diterpene lactones with a rearranged labdane skeleton have been isolated.

For the purposes of systematizing the medicinal plants of Armenia, we have begun a chemical investigation of the lactones of the previously unstudied species T. hyrcanicum L. (Hyrcanian germander). From an acetone extract of the plant by chromatography on alumina we isolated four new diterpenoids which we call, by analogy with teucrins A-F [8], teucrins H1-4. Teucrins H1 and H4 are similar in composition and spectra properties to teucrin A, and teucrin H2 to teucrin E. A direct comparison of the melting points of mixtures and the results of chromatography of the substances that we had isolated with samples of teucrins A and E* showed that they were different.

Teucrin H1 (I) is the main component of the total lactones has the composition $C_{19}H_{20}O_6$, and contains a furan ring (coloration with Ehrlich's reagent, and absorption at 3170, 3150, 1605, 1510, and 880 cm^{-1}) that is substituted in the β position (2H, multiplet at 7.43 ppm, and 1H, multiplet at 6.35 ppm; Fig. 1), a hydroxy group (3510 cm^{-1}), and two γ-lactone rings. One lactone is saturated (1770 cm^{-1}), and the other is α,β-unsaturated (1745 cm^{-1} and 217 nm). The presence of two lactone rings is also confirmed by the reaction of (I) with two equivalents of alkali.

A hydroxy group of (I) has a secondary alcoholic nature, as is shown by a downfield shift in the PMR spectra of the signal of the proton geminal to the hydroxy group on acetylation (shift of the broadened one-proton singlet from 4.60 to 5.70 ppm). The spectrum of the acetate contains, in place of the signal of the proton of the hydroxy group (1H, multiplet at 3.38 ppm) the signal of the methyl radical of an acetyl group (3H, singlet at 2.1 ppm). The presence in (I) of a hydroxy group and the position of its signal in the PMR spectrum were confirmed by deuterium exchange.

The molecular composition of the dilactone (I) and the presence in it of a β-substituted furan ring and a secondary methyl group (3H, doublet, J = 7 Hz at 1.35 ppm) enables us to suggest a structural closeness of (I) to teucrin A (II) [8, 9] and, consequently, a biogenetically expected relationship of (I) to the rearranged norlabdane derivatives. The mass and PMR spectra of (I) and its acetate agree well with this hypothesis. In the mass spectrum of (I) there are strong peaks of ions with m/e 95, 94, and 81 which are characteristic for furolactones containing the lactone oxygen in the allyl position to the furan ring [7, 8, 11].

*Samples of teucrins A and E were kindly provided by D. P. Popa.

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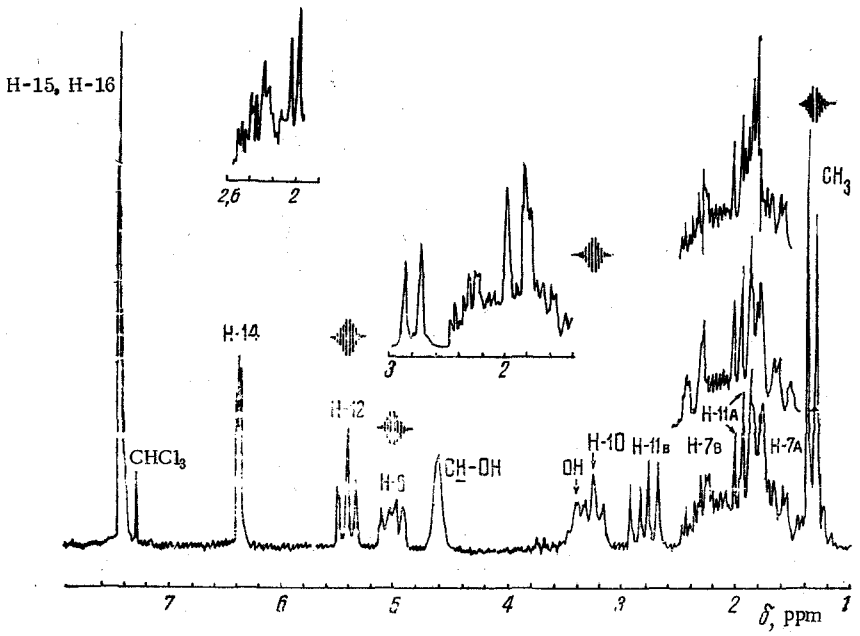
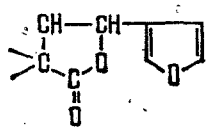


Fig. 1. PMR spectrum of teucrin H1 (100 MHz).

The signal of the proton geminal to this lactone oxygen appears in the PMR spectrum of (I) in the form of a triplet at 5,40 ppm (ABX system with $J_{AX}+J_{BX}=16$ Hz), i.e., there are the two protons of a methylene group in the vicinal position to this proton. The methylene group appears as two one-proton quartets at 2.2 and 2.8 ppm, which is shown by double resonance. These facts prove the presence in (I) of the grouping characteristic for diterpene lactones of the genus Teucrium.

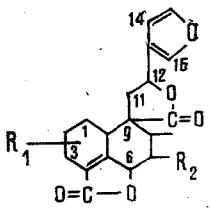


In the weak field at 5.02 ppm, the PMR spectra of (I) and its acetate show a one-proton doublet of doublets forming the X part of a ABX system. Double resonance has shown that the AB part of the system consists of two one-proton multiplets (octets) at 2.36 and 1.6 ppm which acquire the form of quartets when the signal at 5.02 ppm is saturated. The signal at 5.02 ppm corresponds, in all probability, to a proton geminal to the oxygen of the second, α,β-unsatutated, lactone ring, and in the position vicinal to it there are only the two protons of a methylene group.

The absence of signals of olefinic protons indicates tetrasubstitution of the double bond conjugated with the lactone carbonyl, and this is confirmed by the presence of absorption at 1700 cm^{-1} in the IR spectrum of (I) which is characteristic for α,β-unsaturated γ-lactones [8, 12].

It was shown by the method of double resonance with irradiation of the signal of the methyl group at 1.35 ppm that the methine proton geminal to the methyl group appears in the form of a broad multiplet with its center at 2.26 ppm. A one-proton triplet at 3.26 ppm ($J_1+J_2=14$ Hz) must be ascribed to the methine proton at C_{10} of a rearranged labdane skeleton and, thus, it must be assumed that position 1 is not occupied by substituents. The signals of the protons of the C_1 methylene group are observed in the form of a multiplet in the 1.7-1.95 ppm region by double resonance on saturation of the signal of the C_{10} proton.

The facts given enable us with high probability to propose for teucrin H_1 a molecular sketeton identical with that of teucrin A (II) and to put forward its structure in the form of formula (I).



I. R_1=OH; R_2=H
II. R_1=H; R_2=O

Teucrin H2, $C_{20}H_{24}O_6$, is a compound which it is difficult to separate chromatographically from teucrin H_1. It contains a secondary methyl group (3H, 1.0 ppm), and a readily acetylated secondary alcoholic hydroxy group (3540 cm^{-1}). The IR spectrum of the acetate $C_{21}H_{22}O_7$, lacks the absorption of a hydroxy group but has the band of an acetyl carbonyl at 1740 cm^{-1}. Acetylation leads to a shift in the signal of the proton geminal to the hydroxyl from 3.7 to 4.9 ppm and to the appearance of a three-proton singlet of an acetyl group at 2.1 ppm. Teucrin H2 contains two saturated γ-lactone rings (1760 cm^{-1}) and absorbs two equivalents of alkali. Just like teucrin H1, according to its spectra it contains a β-substituted furan ring, and in its PMR spectrum there are the signals of the $C_{11}-C_{12}$ protons of the ABX system of the furolactone moiety of (I) and of teucrin A. Furthermore, a two-proton AB system is observed in the 4.5 ppm region which is due to an isolated methylene group. The facts given show the closeness of the structure of teucrin H2 and E [8].

Teucrin H3, $C_{20}H_{20}O_7$, is a monoacetate, as is shown by a 3H singlet at 2.06 ppm in the PMR spectrum and by the ion M − 42 in the mass spectrum. The IR spectrum of this compound lacks the absorption of a hydroxyl but there is absorption at 1730 cm^{-1} (carbonyl of an acetyl group) and absorption bands of a β-substituted furan and of a saturated γ-lactone [1760, 1750 (shoulder) cm^{-1}]. The substance reacts with three equivalents of alkali.

Teucrin H4, $C_{19}H_{20}O_6$, is isomeric with teucrin H1. It contains a saturated and an unsaturated γ-lactone ring, one readily acetylated hydroxy group and a β-substituted furan ring. Teucrin H4 is the most polar of the four lactones isolated.

EXPERIMENTAL

The melting points were determined on a Boetius instrument. Thin-layer chromatography (TLC) was performed on a fixed layer of type KSK silica gel. These spots were revealed with 50% sulfuric acid, Ehrlich's reagent [7], or iodine vapor. The IR spectra were taken on a UR-20 spectrometer in KBr, the PMR spectra on a Varian 60A spectrometer and a JNM-4H-100/100 MHz instrument with TMS as internal standard in $CDCl_3$ solution, the mass spectra on a MKh-1303 instrument at 95°C with an ionization energy of 40 eV, and the UV spectra on a Specord UV-VIS instrument in ethanol.

The analyses of all the compounds corresponded to the calculated figures. The number of lactone rings was determined from the absorption of alkali by the method of Barton and Elad [13].

Isolation of the Diterpene Lactone. To choose the optimum conditions for the isolation of the lactone fraction we performed preparative extraction with various solvents of the air-dry comminuted raw material collected in the flowering-fruit-bearing phase on July, 29-30, 1974, in the region of Shikakhokh-Tsav, Armenian SSR:

a) 200 of the raw material was exhaustively extracted with cold acetone until Ehrlich's reaction on TLC was negative. After the solvent had been distilled off, the residue was dissolved in 80% aqueous acetone and was freed from plant pigments [14]. The acetone was distilled off and the residue was extracted with chloroform. The chloroform solution was washed with water and dried and the chloroform was distilled off. The yield of lactone fraction was 4.82 g (2.41%);

b) 200 g of the raw material was extracted with methanol in the cold. The extract was concentrated in vacuum to a volume of 50 ml, diluted with 25 ml of water, and filtered from ballast substances. The aqueous methanolic solution was extracted successively with benzene and chloroform. The benzene extract was freed from pigments. The two extracts were washed with water and dried and the solvents were distilled off. The yield of lactone fraction was 4 g (2%), of which 2.55 g (1.28%) was obtained from benzene extract, and 1.48 g (0.74%) from the chloroform extract;

c) By aqueous extraction [15], 200 g of raw material yielded 3.82 g (1.9%) of lactone fraction.

The lactone composition of all the extracts obtained by the three methods were shown to be identical by TLC in the chloroform−methanol (19:1) system. The higher yield and mild temperature conditions enabled us to give preference to the acetone extraction method (a).

From 18 kg of raw material by acetone extraction we obtained 305 g of an aromatic light brown resin, of which 51.8 g was chromatographed on 1.9 kg of aluminum (activity grade III, V). On elution with a mixture of chloroform and ether (90 : 10), teucrins H3, H2, and H1 were isolated successively. Teucrin H1 was also eluted by a mixture of chloroform and ether (95:5), and pure chloroform eluted teucrin H4.

Teucrin H1. Colorless needles (3.1 g), $C_{19}H_{20}O_6$, mp 180-182°C (ethanol), $[\alpha]_D^{20}$ −95 ± 3.5° (c 0.75; chloroform), R_f^* 0.68; $R_f^\dagger$ 0.13 [here and below, * denotes the chloroform−methanol (19:1) system, and † the chloroform−ether (17:3) system]. IR spectrum, cm^{-1}: 3510, 3170, 3150, 1770, 1745, 1700, 1605, 1510, 1460, 1340, 1280, 1180, 1160, 1090, 1030, 880. UV spectrum: λ_{max} 204, 208, 217 nm (log ε 4.43; 4.42; 4.28).

Mass spectrum (main peaks): 344 (M^+), 326, 316, 308, 298, 281, 280, 232, 222, 221, 178, 176, 109, 105, 95, 94, 81, 79, 57, 55, 45, 44, 43.

Teucrin H2. Colorless prismatic crystals (2.2 g), $C_{20}H_{24}O_6$, mp 212-214°C (ethanol); $[\alpha]_D^{26}$ −12 ± 4° (c 0.68; chloroform), R_f^* 0.69; $R_f^\dagger$ 0.19.

IR spectrum, cm^{-1}: 3540, 3180, 3160, 1760, 1605, 1515, 1480, 1360, 1325, 1250, 1195, 1170, 1720, 1155, 1030, 995, 880.

UV spectrum: λ_{max} 206, 207 nm (log ε 4.5, 4.45). Mass spectrum: 360 (M^+), 342, 332, 324, 310, 286, 266, 248, 235, 222, 220, 203, 178, 161, 159, 147, 145, 133, 131, 123, 119, 111, 109, 105, 95, 94, 81, 71, 69, 67, 57, 55, 44, 43.

Teucrin H3. Colorless crystals (1.3 g), $C_{20}H_{20}O_7$, mp 216-218°C (chloroform), $[\alpha]_D^{26}$ +89 ± 4°C (c 0.65; chloroform), R_f^* 0.79; $R_f^\dagger$ 0.22.

IR spectrum, cm^{-1}; 3170, 3145, 3110, 1760, 1750 (shoulder), 1730, 1650, 1605, 1510, 1390, 1340, 1270, 1230, 1195, 1170, 1030, 935, 880.

UV spectrum: λ_{max} 206, 207 nm (log ε 4.42, 4.36).

Mass spectrum: 372 (M^+); 330, 311, 301, 293, 285, 283, 267, 255, 253, 236, 220, 218, 207, 203, 185, 178, 173, 163, 161, 145, 134, 133, 121, 109, 105, 95, 94, 81, 79, 71, 69, 67, 57, 55, 53, 43, 42.

Teucrin H4. Colorless needles (0.22 g), $C_{19}H_{20}O_6$, mp 225-226°C (ethanol), $[\alpha]_D^{26}$ +48 ± 4° (c 0.66; ethanol), R_f^* 0.33; $R_f^\dagger$ 0.05.

IR spectrum; cm^{-1}: 3350, 3480 (assoc.), 3150, 1770, 1740, 1700, 1600, 1505, 880.

UV spectrum: λ_{max} 205, 210, 218 nm (log ε 4.32, 4.27, 4.19). Mass spectrum: 344 (M^+), 326, 316, 315, 308, 300, 298, 282, 281, 250, 232, 222, 221, 178, 176, 173, 109, 105, 95, 94, 81, 79, 69, 67, 57, 55, 45, 44, 43.

Acetylation of Teucrins H. A solution of 100 mg of the substance in 2 ml of pyridine was treated with 1 ml of acetic anhydride, and the mixture was left at room temperature for 2 days. Then it was poured into 50 g of ice and the precipitate that deposited was filtered off. After recrystallization from ethanol, the corresponding acetate was obtained.

1. Acetate of teucrin H1, yield 87 mg, $C_{21}H_{22}O_7$, mp 162-164°C, R_f^* 0.86, $R_f^\dagger$ 0.35. IR spectrum: 3160, 1760, 1750, 1735, 1700, 1600, 1505, 1245, 1180, 880. M^+ 386 (mass spectrum).

2. Acetate of teucrin H2, yield 100 mg, $C_{22}H_{26}O_7$, mp 199-201°. R_f^* 0.93; $R_f^\dagger$ 0.33. IR spectrum: 3160, 3140, 1770, 1740, 1600, 1510, 1250, 1120, 880. M^+ 402 (mass spectrum).

3. Acetate of teucrin H4, yield 92 mg, $C_{21}H_{22}O_7$, mp 211-212°C. R_f^* 0.71; $R_f^\dagger$ 0.32. IR spectrum: 3150, 1760, 1745, 1735, 1700, 1600, 1510, 1250, 880. M^+ 386 (mass spectrum).

SUMMARY

Four new diterpene lactones have been isolated from the plant Teucrium hyrcanicum L. (Hercanium germander) and have been named teucrins H1-4. On the basis of spectral characteristics, a probable structure has been proposed for teucrin H1, which has been assigned to the rearranged norlabdane derivatives.

LITERATURE CITED

1. S. Ya. Zolotnitskaya, Medicinal Resources of the Flora of Armenia [in Russian], Vol. 2, Erevan (1965), p. 238.
2. K. Neumann, Planta Medica, 13, No. 3, 331 (1965).
3. A. Vrgoč, Chemist and Druggist, 124, 706 (1936).
4. J. Petricič, Acta Pharm. Yugoslav., 2, 29 (1952).
5. D. Iordanov and N. Nikolov, Phytotherapy [in Bulgarian], Sofia (1970), p. 268.
6. S. E. Zemlinskii, Medicinal Plants of the USSR [in Russian], Moscow (1958), pp. 401-402.

7. C. H. Briskorn and T. Pfeufer, Chem. Ber., 100, 1998 (1967).
8. D. P. Popa and A. M. Reinbol'd, Khim. Prirodn. Soedin., 67 (1972); 31 (1973); 321, 589 (1974).
9. D. P. Popa, A. M. Reinbol'd, and A. I. Rezvukhin, Khim. Prirodn. Soedin., 169 (1973).
10. E. Fugita, I. Ichida, T. Fugita, N. Masaki, and K. Osaki, J. Chem. Soc. Chem. Comm., 20, 793 (1973).
11. H. Budzikiewiez, C. Djerassi, and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, Holden-Day, San Francisco, Vol. 2 (1964).
12. L. Novotny, J. Jizba, V. Herout, F. Sorm, L. H. Zalkow, S. Hu, and C. Djerassi, Tetrahedron, 19, 1101 (1963).
13. D. H. R. Barton and D. Elad. J. Chem. Soc., 2085 (1956).
14. G. V. Lazur'evskii, I. V. Terent'eva, and A. A. Shamshurin, Practical Work in the Chemistry of Natural Compounds [in Russian], Moscow (1966), p. 283.
15. K. S. Rybalko, N. N. Ban'kovskaya, and R. I. Evstratova, Med. Prom. SSSR, No. 3, 13 (1962).

SPECTROPHOTOMETRY OF GLYCOSIDES OF OLEANOLIC ACID AND HEDERAGENIN IN CONCENTRATED SULFURIC ACID

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In recent years, investigations of triterpene glycosides have been performed on a broad scale. The structures of about 200 such compounds have been demonstrated. Glucosides of oleanolic acid and of hederagenin have proved to be the most widely distributed – they make up more than half the compounds discovered. Some triterpene glycosides have found use in medicine. In view of this, the development of methods for their analytical determination in preparations, medicinal forms, and the plant raw material is a matter of considerable interest.

Triterpenoids with an isolated double bond have an absorption maximum in the UV region of the spectrum in the range between 193 and 205 nm [1]. We have previously [2] made use of this property for determining the molecular weights of glycosides of hederagenin and gypsogenin. Unfortunately, this region of the spectrum is unsuitable for the quantitative determination of triterpenoids and their glycosides. Consequently, in the present work we have investigated the possibility of determining triterpene glycosides spectrophotometrically in concentrated sulfuric acid.

The halochromic reaction in concentrated sulfuric acid has been widely studied for a whole series of natural compounds. It has been used for the identification and quantitative analysis of steroid and triterpenoid sapogenins [3-13]. With concentrated sulfuric acid, the latter show characteristic absorption peaks in the UV region of the electronic spectrum which can be used for analytical determination [3, 10, 12]. It has been found that to obtain reproducible results the main reaction conditions – the concentration of the sulfuric acid, the time (rate) of the reaction, and the temperature – must be strictly controlled.

At the same time for a long period there was no single opinion among the authors making use of the halochromic reaction in sulfuric acid concerning the conditions for performing this reaction: some performed it at room temperature [14, 15] and others [4, 10, 11, 13] recommended thermostating at 40, 60, 70°C, etc. There was no agreement on the time of performance of the reaction, either.

V. F. Semenchenko et al. [11] studied the reaction of triterpenoids with concentrated sulfuric acid and, making use of the method of mathematical planning, concluded that the optimum conditions for this reaction are heating the reaction mixture at 70°C for 60 min. Karting et al. [13] came to the same conclusion.

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