# STRUCTURE OF STACHYSIC ACID – A NEW DITERPENOID OF THE KAURANE SERIES

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Continuing work on the higher isoprenoids of plants of the family Labiatae, we have investigated extracts of Stachys silvatica L. (whitespot betony) – a plant related to the S. annua L. studied previously, which contains diterpenoids with a rearranged labdane carbon skeleton [1]. Substances of related structure were expected here. However, on analysis of both petroleum-ether and of acetone extracts of the raw material, as the main product we isolated a crystalline substance with the composition $C_{22}H_{32}O_4$ which, according to the chemical and spectral investigations described below, is a previously unknown acid of the kaurane series, and we have called it stachysic acid (I).

This is one of the few cases of the isolation of kaurane diterpenoids from plants of the family Labiatae. Hitherto, there have been only two genera – Sideritis and Isodon – from which such compounds have been isolated [2, 3].

The IR spectra of compound (I) showed the maxima characteristic for an acetate group (1730 and 1240 $cm^{-1}$), carboxyl (1700 $cm^{-1}$), and an exocyclic double bond (1660 and 880 $cm^{-1}$). It was soluble in an aqueous solution of alkali, and on methylation with diazomethane gave a methyl ester (II) containing no free hydroxy groups. The alkaline hydrolysis of (I) gave an acid (III) the methyl ester (IV) of which contained one readily acetylatable hydroxy group. Oxidation of the ester (IV) gave a ketone (V), and catalytic hydrogenation of the latter gave an alcohol (XIV) which was converted on oxidation into the ketone (VI).

The NMR spectrum of the hydroxy ester (IV) shows the signals of two tertiary methyl groups (0.80 and 1.28 ppm), the latter of which relates to a methyl group geminal to the methoxycarbonyl group. The signal of an ester methyl is observed at 3.48 ppm and a broad multiplet of two vinyl protons, at 4.53 ppm. All this gave grounds for assuming that stachysic acid belongs to the tetracyclic diterpenoids of the kaurane group.

In order to confirm the proposed carbon skeleton and to show the position of the double bond in the molecule and the stereochemistry of the ring linkage, a correlation of the acid (I) with (–)-kaurene (XII) was performed. The reduction of (I) with lithium tetrahydroaluminate in tetrahydrofuran gave a diol (VII) which was converted by reaction with p-toluenesulfonyl chloride into the tosylate (VIII). Reduction of the latter with lithium tetrahydroaluminate gave a mixture of substances from which, by chromatography, we isolated (–)-kaurene (XII), an alcohol (IX) identical with the product obtained from corymbol [4], and the initial diol (VII). The alcohol (IX) was readily oxidized with chromium trioxide in pyridine to the ketone (X), which, after reaction with ethanedithiol and subsequent desulfuration of the thioketal with Raney nickel, was also converted into (–)-kaurene (XII), identical, according to its IR spectrum, melting point, and specific rotation, with a known sample.

The position of the carboxy group in the diterpenoid (I) was established by a comparison of the NMR spectra of compounds (IV) and (IX). In the spectrum of the latter, the signals of the two geminal methyl groups are present in a stronger field (0.93 and 0.98 ppm) than the signal of the $C_4$-methyl group in the spectrum of compound (IV) (1.28 ppm). This shows that the methoxycarbonyl group in the ester (IV) and, consequently, the carboxy group in stachysic acid (I) must be present at carbon atom 4.

The position of the acetoxy group in substance (I) or the hydroxy group in its derivatives (IV) and (IX) at $C_6$ was shown by the NMR spectra of the keto ester (VI) using the solvent effect [5].

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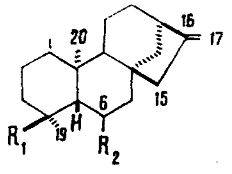
For the $H_{17}$ and $H_{20}$ protons the signs of the differences $\Delta\delta_{CCl_4} - \delta_{C_6H_6}$ are positive (0.03 and 0.13 ppm, respectively), and for $H_{19}$ this difference is negative (−0.15 ppm). This is possible only if the first two methyl groups are behind a plane passing through the carbonyl carbon atom and perpendicular to the direction of the C = O bond, and the third methyl is in front of it. In kaurane derivatives, this plane can only be a plane passing through $C_6$.

The value of Δ for the $H_{19}$ singlet in compound (VI) corresponds to an axial methyl group in the β-position to a carbonyl group. Consequently, the carboxy group in stachysic acid is equatorial. This is also confirmed by the position of the signal of the two $H_{18}$ protons, forming an AB system [6, 7] the center of which is at 3.30 ppm in the spectrum of the diol (VII) and 4.04 ppm in the spectrum of its acetate (XIII).

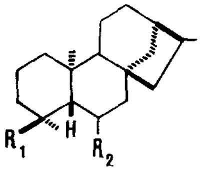
What has been said above provides grounds for considering that stachysic acid is 6-β-acetoxy-(−)-kaur-16-en-18β-oic acid (I).

From the neutral fractions of the extract of the plant under investigation we isolated two other diterpene compounds, alcohols, identical according to melting points, mixed melting points, IR spectra, and TLC with 6-hydroxy- and 6,8-dihydroxy-(−)-kaurenes (IX and VII), which were also obtained from the acid (I).



I − V, VII − X, XII, XIII

I. $R_1 = COOH$, $R_2 = \beta - OAc$
III. $R_1 = COCH$, $R_2 = \beta - OH$.
V, VI. $R_1 = COOCH_3$, $R_2 = = O$.
VIII. $R_1 = CH_2OTs$ $R_2 = OTs$.
X, XI. $R_1 = CH_3$, $R_2 = = O$.
XIII. $R_1 = CH_2OAc$, $R_2 = \beta - OAc$



VI, XI, XIV

II. $R_1 = COOCH_3$, $R_2 = \beta - OAc$
IV. $R_1 = COOCH_3$, $R_2 = \beta - OH$.
VII. $R_1 = CH_2OH$, $R_2 = OH$.
IX. $R_1 = CH_3$, $R_2 = OH$.
XII. $R_1 = CH_3$, $R_2 = H$.
XIV. $R_1 = COOCH_3$, $R_2 = OH$.

## EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrometer, the NMR spectra on an RS-60 instrument (working frequency 60 MHz, internal standard tetramethylsilane) in chloroform, and the mass spectra on an MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source. The melting points of the substances were determined on a Kofler block. KSK silica gel was used for chromatography. The analyses of all the compounds corresponded to the calculated figures.

Extraction with Petroleum Ether. The epigeal part of the plant collected in the flowering stage (2 kg) was dried in the air, comminuted, and extracted three times with petroleum ether (bp 70-100°C) with heating on the water bath for 8 h. The solvent was evaporated to 0.5 liter. A precipitate (3.5 g) of the acid (I) with the composition $C_{22}H_{32}O_4$ deposited. After two crystallizations from methanol, mp 242-243°C, $[\alpha]_D$ −66° (c 4.06; pyridine). IR spectrum (KBr), $cm^{-1}$: 3090, 1660, 880 (exocyclic double bond), 1690 (carboxyl), 1735 and 1240 (acetate).

The methylation of (I) with diazomethane gave methyl stachysate, mp 156-158°C (from petroleum ether). IR spectrum ($CCl_4$), $cm^{-1}$: 1740, 1730, 1660, 1250, 880.

Reaction of (I) with Alkali. A mixture of 200 mg of the substance with 5 ml of methanol containing 8% of KOH was boiled for 1 h. The solvent was evaporated off, and the residue was treated with ether. The yield of the hydroxy acid $C_{20}H_{30}O_3$ (III) was 190 mg, mp 216-218°C (from ethyl acetate).

The methylation of (III) with diazomethane gave the methyl ester $C_{21}H_{32}O_3$ (IV), mp 150-152°C (from petroleum ether−benzene). IR spectrum ($CCl_4$), $cm^{-1}$: 3570 (hydroxyl), 3090, 1660, 880 (double bond), 1715, 1250 (methyl ester). NMR spectrum, ppm: 0.80, 1.28, and 3.48 (singlets, 3H each, methyls at $C_{10}$ and $C_4$ and ester methyl, respectively), 4.53 (2H, broadened singlet, protons of a double bond).

Extraction with Acetone. The dry comminuted plant (2 kg) was extracted with acetone by steeping at room temperature. Evaporation of the solvent left 50 g of extract, from which the plant waxes (15.2 g) were separated by precipitation in methanol (300 ml). Then the methanol was distilled off, the residue was dissolved in ether, and the solution was washed five times with 5% caustic soda solution to eliminate products of an acid nature. The ethereal layer was washed with water, dried with sodium sulfate, and evaporated. The yield of neutral fraction was 12.6 g. The alkaline solutions were acidified with hydrochloric acid, the precipitate that separated out was taken up in ether, and the solution was washed, dried, and evaporated. This gave 22 g of acid fraction.

The neutral fraction (5 g) was chromatographed on 90 g of silica gel. A mixture of petroleum ether and benzene (7 : 3) eluted 1.28 g of phytol, identical with a known sample according to IR spectra and TLC. Petroleum ether containing 50% of benzene eluted 0.18 g of the alcohol (IX), identical with that obtained from stachysic acid. Then benzene eluted 1.3 g of $\beta$-sitosterol, and benzene–ether (7 : 3) eluted 0.53 of the diol (VII), identical with the product of the reduction of stachysic acid with lithium tetrahydroaluminate.

The acid fraction of the extract (22 g) was dissolved in ether, and the solution was washed with saturated sodium bicarbonate solution and with water and was dried and evaporated. The residue (15.2 g) was methylated with diazomethane in ether, and was then chromatographed on 440 g of silica gel. A mixture of ether and benzene (4 : 1) eluted 4 g of a liquid fraction in which, after chromatography on alumina, equal amounts of methyl octadec-4-enoate and methyl stearate were found, these being identified by comparison with authentic samples (IR, TLC). Benzene eluted 5.2 g of the methyl ester (IV) described above.

The Ketone (V). The methyl ester (IV) (140 mg) was oxidized with chromium trioxide in pyridine at room temperature for 12 h. After the usual working up, 120 mg of the keto ester $C_{21}H_{30}O_3$ (V) with mp 122-123°C (from petroleum ether) was obtained. IR spectrum ($CCl_4$), $cm^{-1}$: 1740 (ester), 1715 (ketone), 1655 and 885 (double bond); $M^+$ 330 (mass spectrometry).

The Saturated Ketone (VI). At 18°C and 750 mm, 110 mg of the ester (IV) in 5 ml of acetic acid in the presence of 20 mg of platinum oxide was saturated with hydrogen, whereupon it absorbed 7.4 ml of hydrogen, corresponding to 1 meq. After the usual working up, 100 mg of the dihydro derivative $C_{21}H_{34}O_3$ (XIV) was isolated.

The oxidation of compound (XIV) with chromium trioxide in pyridine gave 90 mg of the keto ester (VI), mp 112-113°C (from petroleum ether). IR spectrum ($CCl_4$), $cm^{-1}$: 1740 (ester), 1718 (ketone). NMR spectrum in $CCl_4$, ppm: 1.0 (J = 6 Hz), 1.05, 1.23, 3.48; in benzene: 0.97 (J = 6 Hz), 0.92, 1.38, and 3.10 (the methyls at $C_{16}$, $C_{10}$, and $C_4$ and the ester methyl, respectively).

Reduction of Compound (I) with Lithium Tetrahydroaluminate. A mixture of 300 mg of the substance and 500 mg of lithium tetrahydroaluminate in 8 ml of tetrahydrofuran was boiled under reflux for 5 h. After the usual working up, 280 mg of a diol $C_{20}H_{32}O_3$ (VII) was obtained with mp 182-184°C (from ethyl acetate), $[\alpha]_D$ −60° (c 4.7; chloroform), $M^+$ 304 (mass spectrum), NMR spectrum, ppm: 0.93, 1.15 (singlets, 3H each, methyls), 3.30 (unresolved multiplet, 2H, $H_{18}$ protons), and 4.57 (2H, vinyl protons).

The acetylation of 100 mg of the diol (VII) with acetic anhydride and the chromatography of the reaction product yielded 90 mg of the acetate (XIII), mp 98-100°C (from petroleum ether). NMR spectrum, ppm: 0.97 and 1.0 (singlets, 3H each, methyl groups), 1.99 and 1.97 (two singlets, 3H each, acetate methyls), 3.90 and 4.18 (2H, AB, J = 17 Hz), and 4.52 (2H, vinyl protons).

Preparation of (IX). With stirring and cooling to −5°C, a solution of 200 mg of the diol (VII) in 3 ml of pyridine was added to a solution of 1 g of p-toluenesulfonyl chloride in pyridine. The mixture was kept at 0°C for 14 h. After the usual working up, 244 mg of the tosylate (VIII) was isolated, and this was reduced by being heated with 300 mg of lithium tetrahydroaluminate in tetrahydrofuran for 5 h. The reaction product was chromatographed on silica gel. Benzene eluted 40 mg of a crystalline substance with mp 50°C (from methanol), identical according to its IR spectrum with (−)-kaurene (XII). A mixture of benzene and 1.5% of methanol eluted 100 mg of (IX), composition $C_{20}H_{32}O$, mp 155-157°C (from a mixture of petroleum ether and benzene). IR spectrum (tablets with KBr), $cm^{-1}$: 1660 and 880 (double bond). NMR spectrum, ppm: 0.75, 0.93, and 0.98 (singlets, 3H each, methyl groups), 4.53 (broadened singlet, 2H, vinyl protons). A mixture of benzene and 6% of methanol eluted 70 mg of the initial diol (VII).

Conversion of the Alcohol (IX) into ( − )-Kaurene (XII). The alcohol (IX) (240 mg) was oxidized with chromium trioxide in pyridine at room temperature for 12 h. After the usual working up and chromatography on silica gel in benzene, 110 mg of the ketone (X) was obtained with mp 106-107°C (from ethyl acetate), and on reaction with 0.5 ml of ethanedithiol in 4 ml of absolute ether in the presence of three drops of boron trifluoride etherate for 3 h at 0°C this formed 190 mg of the thioketal. The latter was desulfurated with deactivated Raney nickel by boiling in dioxane for 5 h. The solvent was evaporated, the residue was diluted with water and extracted with ether, and the ethereal extract was washed with water, dried with sodium sulfate, and evaporated. This gave 50 mg of a crystalline product which was chromatographed on 2.5 g of silica gel impregnated with $AgNO_3$. A mixture of petroleum ether and 5% of benzene eluted 20 mg of a crystalline substance with mp 50°C (from methanol), $[\alpha]_D$ −70° (c 6.2, chloroform), identical according to its IR spectrum with (−)-kaurene (XII).

## SUMMARY

In addition to 6-hydroxy- and 6,18-dihydroxy-(−)-kaurenes, from Stachys silvatica L. (family Labiatae) we have isolated a new diterpenoid of the kaurane series which we have called stachysic acid and for it we have established the structure of 6β-acetoxy-(−)-kaur-16-en-18β-oic acid.

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