STRUCTURE OF STACHYSOLONE

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The isolation from Stachys annua L., family Labiatae, of three bitter acetates of a new diterpene keto diol, called stachysolone, has been reported previously [1]. We now give a proof of the structure of this compound.

It was established on the basis of IR and UV spectra [2] that the diterpenoid (I) is an α, β-unsaturated ketone forming on heating with acetic anhydride in pyridine mono- and diacetates (II and III) identical with the corresponding natural compounds. It is not oxidized by sodium periodate but is readily oxidized by chromium trioxide in pyridine, forming keto derivatives (IV), which shows the presence in the molecule of one secondary and one tertiary hydroxy group not in the vicinal positions. The hydrogenation of stachysolone in ethyl acetate over palladium gave tetrahydrostachysolone (V). The dehydrogenation of stachysolone with selenium led to the isolation of 1,2,5-trimethylnaphthalene.

The NMR spectrum of stachysolone (Fig. 1a) has the signals of five methyl groups: three tertiary at 1.07, 1.21, and 1.41 ppm (singlets each with an intensity of 3H), one secondary at 0.98 ppm (doublet, 3H, J = 6.8 Hz), and one group of the $CH_3-C=CH-$type at 1.94 ppm (narrow doublet, 3H, J = 1.2 Hz). In addition signals are recorded (Table 1) of three protons at 5.01, 5.18, and 5.86 relating to a vinyl group, one proton of the CH–OH type at 3.95 ppm, and two protons of hydroxy groups – secondary (doublet at 4.50 ppm, J = 3.5 Hz) and tertiary (singlet at 4.42 ppm), which is confirmed in the presence of traces of CF_3COOH.

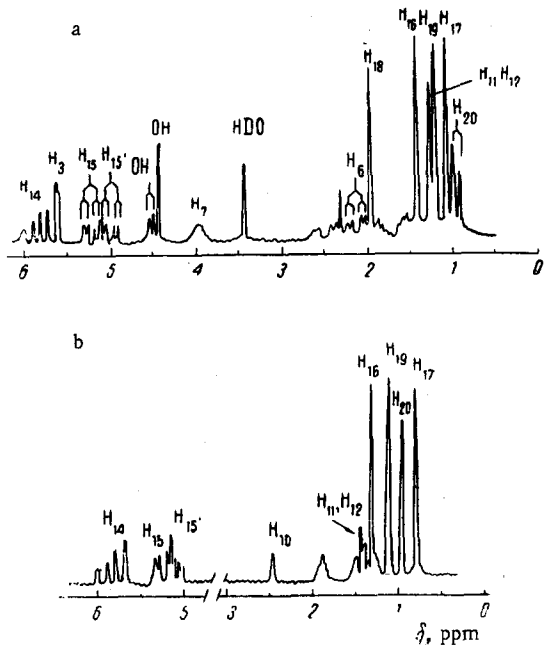

Fig. 1. NMR spectra of stachysolone (I) (a) and of deuterooxostachysolone (XII) (b).

The above facts, and also the absence from the IR spectrum of the doublet for a gem-dimethyl group (at 1375 cm^{-1}) enabled us to assign stachysolone to the diterpenoids with a rearranged hydrocarbon skeleton of labdane – of the type of kolavane (VI) – with a 14.15 double bond and a tertiary hydroxyl at C_{13}.

Analysis of the mass-spectrometric results confirmed the hypotheses discussed. In the mass spectrum of stachysolone there are the peaks of ions appearing as a result of the gradual fragmentation of the side chain (M–27, M–70, M–85, and M–99 m/e), which is analogous to the fragmentation of labdane derivatives with the same side chain [3] and is also in agreement with the presence in the NMR spectrum of (I) of a four-proton singlet at 1.26 ppm assigned to the two methylene groups of the $C_{11}-C_{12}$ link. The ion M–99 m/e corresponds to the bicyclic system of the molecule and readily eliminates water with the formation of a fragment with m/e 203 (M–99–18). In the spectrum of tetrahydrostachysolone (V), in place of this ion there is an ion with m/e 205. This shows that the bicyclic nucleus of the stachy-

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TABLE 1. Chemical Shifts and Spin–Spin Coupling Constants in the NMR Spectra of Stachysolone (I) and Oxostachysolone (IV)[a]

Compound	Solvent	H_3	H_6	$H_{6'}$	H_7^b	H_{14}	H_{15}	$H_{15'}$	H_{16}^c	H_{17}^c	H_{18}	H_{19}^c	H_{20}
Id	D_6 – DMSO	5,62	2,15 $J_{6,7}=2,5$ $J_{6,6'}=13,8$	–	3,95	5,86 $J_{14,15}=17,4$ $J_{14,15'}=10,6$	5,18 $J_{15,14}=17,4$ $J_{15,15'}=2,3$	5,01 $J_{15',14}=10,6$ $J_{15',15}=2,3$	1,41	1,07	1,94 $J_{3,18}=1,2$	1,21	0,98 J=6,8
IV	$CDCl_3$[e]	5,78	–	–	–	5,87 $J_{14,15}=17,5$ $J_{14,15'}=10,5$	5,21 $J_{15,14}=17,5$ $J_{15,15'}=1,5$	5,08 $J_{15',14}=10,5$ $J_{15',15}=1,5$	1,29	0,79	1,87 $J_{3,18}=1,2$	1,09	0,94 J=6,5
IV	C_6D_6[e]	5,61	2,17 $J_{6,6'}=12$	1,78[f] $J_{6',6}=12$	–	5,70 $J_{14,15}=17,5$ $J_{14,15'}=10,5$	5,22 $J_{15,14}=17,5$ $J_{15,15'}=1,5$	4,95 $J_{15',14}=10,5$ $J_{15',15}=1,5$	1,09	0,51	1,43 $J_{3,18}=1,2$	0,71[f]	0,86 J=6,5

[a]The chemical shifts (ppm) are given on the δ scale relative to TMS and the spin–spin coupling constants are given in Hz.
[b]The half width of the signal after the addition of traces of trifluoroacetic acid, $W_{1/2} \approx 9$ Hz.
[c]The signal for H_{19} was chosen on the basis that $W_{1/2}$ for $H_{19} > W_{1/2}$ for H_{16} or H_{17}.
[d]The protons at C_{11} and C_{12} form a singlet at 1.26 ppm (4H).
[e]Relative to HMDS with recalculation to the TMS scale, using $\delta_{HMDS} = 0.06$ ppm.
[f]$J_{6',19} \neq 0$ (confirmed by double resonance).

solone molecule contains one double bond. Consequently, the α, β-unsaturated ketone and the $CH_3-C=CH-$ fragment indicated by the NMR spectra form a grouping of the type of $CH_3-C=CH-CO-$. This is also shown by the downfield shift of the signal of the vinyl proton at 5.62 ppm (confirmed by double irradiation).

Thus, the carbonyl group can occupy the C_2 or the C_6 position. A final choice was made after the deuteration of stachysolone in an alkaline medium [4, 5] with subsequent treatment of the product with water or ethanol.

According to mass spectrometry, the deuterostachysolone (VII) formed contained six deuterium atoms in the bicyclic system. Its NMR spectrum had all the signals of the protons described for stachysolone with the exception of the protons of the $CH_3-C=CH-$ fragment, which shows the inclusion of four deuterium atoms in this fragment. The other two deuterium atoms can be present only in the α' position to the carbonyl, and this is possible only if this group is located in ring A, at C_2. This hypothesis is confirmed by an analysis of the mass spectra of tetrahydrooxostachysolone (VIII) and its 2-monothioketal (IX). In place of the peak with m/e 124 corresponding to fragment *a* in the spectrum of (VIII) there is a peak with m/e 200 of the analogous fragment c in the spectrum of (IX). In the mass spectrum of the thioketal of tetrahydrostachysolone (X), as in the spectrum of (IX) there are intense peaks with m/e 145 (fragment c), confirming the presence in the hydrogenated derivatives of stachysolone of a $-CO-CH_2-CH-CH_3$ group with a methyl radical in the β position to the carbonyl group.

I $R_1=R_2=OH$ II $R_1=OAc, R_2=OH$ III $R_1=R_2=OAc$ IV $R_1=O, R_2=OH$
V $R_1=OH, R_2=O$ VIII $R_1=R_2=O$ IX $R_1=O, R_2=S$ X $R_1=OH, R_2=S$
XII $R_1=R_3=R_4=H, R_2=OH$ XI $R_1=R_3=R_4=H, R_2=O$ XII $R_1=R_3=R_4=D, R_2=O$

It follows from what has been said that in the bicyclic nucleus of stachysolone only two positions remain for the secondary hydroxyl: C_6 or C_7. To answer this question, we studied the NMR spectra of the oxidation products of (I) – oxostachysolone (IV) and oxodeuterostachysolone (XI). A detailed analysis of the spectra taken in hexadeuterobenzene (see Table 1) with double irradiation showed that both substances contain the

CH_3-C-CH_2-CO- group, which corresponds to an AB system of two protons forming one narrow doublet at 2.17 ppm and one broadened doublet at 1.78 ppm. The broadening of the latter is a consequence of the weak interaction of the corresponding proton with the protons of the methyl group, giving a singlet at 0.71 ppm.

The presence of a grouping of this type shows the position of the carbonyl of oxostachysolone at C_7 and of the tertiary methyl group at C_5. These conclusions are confirmed completely by the results of the deuteration of oxostachysolone. The deuterooxostachysolone (XII) formed contains nine deuterium atoms (mass spectrometry) and in its NMR spectrum (Fig. 1b) in place of the doublet of the secondary methyl group a singlet has appeared.

On the basis of the results obtained it may be concluded that only structure (I) is possible for stachysolone.

EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrometer, the UV spectra on an SF-4 instrument, the NMR spectrum on an HA-Varian-100 instrument in hexadeuterodimethyl sulfoxide (working frequency 100 MHz, internal standard tetramethylsilane), and the mass spectra on an MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source at an evaporation temperature of 110°C and an ionizing voltage of 70 V. The melting points were determined on a Kofler block. The analyses of all the compounds corresponded to the calculated figures.

Dehydrogenation of Stachysolone. A mixture of 1.7 g of stachysolone previously saturated with hydrogen in ethyl acetate in the presence of $Pd/BaSO_4$, and 2 g of selenium powder was heated at 350°C for 40 h. The reaction mixture was ground and extracted with ether, and the extract was filtered. Distillation of the solvent left 0.15 g of a liquid which was chromatographed on 10 g of alumina (activity grade II). Petroleum ether eluted 60 mg of an aromatic hydrocarbon (picrate, mp 137-138°C) identified by its IR and UV spectra in comparison with an authentic sample as 1,2,5-trimethylnaphthalene. A mixture of the picrates gave no depression of the melting point.

Oxidation of Stachysolone with Sodium Periodate. A mixture of 100 mg of stachysolone and 400 mg of sodium metaperiodate in 10 ml of aqueous methanol (1 : 1) was left at room temperature for 40 h. Then it was diluted with water and extracted with methylene chloride. This gave about 100 mg of a substance identical by IR spectra, thin-layer chromatography, and melting point with the initial stachysolone.

Deuteration of Stachysolone. To 0.5 ml of absolute dioxane were added 0.1 ml of deuterium oxide and 10 mg of metallic sodium, and then 15 mg of stachysolone in 0.5 ml of dioxane (with protection from atmospheric humidity). The mixture was stirred for 15 min in a flask with a reflux condenser and was then distilled to dryness. The residue was treated with 0.5 ml of dioxane and 0.1 ml of deuterium oxide. The experiment was repeated five times. The residue after evaporation was dissolved in absolute ether, washed once from alkali with deuterium oxide, and then twice with water. The ether was dried and distilled. This gave 13 mg of deuterostachysolone $C_{20}H_{26}D_6O_3$ (VII), with mp 152-154°C.

Tetrahydrooxostachysolone (VIII). A solution of 100 mg of tetrahydrostachysolone in 1 ml of anhydrous pyridine was added to the complex obtained from 300 mg of chromium trioxide and 2 ml of pyridine, and the mixture was left at room temperature for 2 h. After the usual working up, 70 mg of substance (VIII) was obtained with the composition $C_{20}H_{34}O_3$, mp 108-109°C (from a mixture of petroleum ether and methylene chloride). IR spectrum ($CHCl_3$): 3610 and 3450 cm^{-1} (hydroxyl), 1700 cm^{-1} (inflection), and 1710 cm^{-1} (oxo group).

Tetrahydrooxostachysolone 2-Thioketal (IX). In the usual way, 50 mg of tetrahydrostachysolone thioketal [2] was oxidized with chromium trioxide in pyridine. This yielded 40 mg of a substance with the composition $C_{22}H_{38}O_2S_2$, mp 123-124°C (from a mixture of petroleum ether and benzene). IR spectrum ($CHCl_3$): 3610, 3490 cm^{-1} (hydroxyl), 1705 cm^{-1} (ketone).

Oxodeuterostachysolone (XI). Deuterostachysolone (VII) (30 mg) was oxidized with chromium trioxide in pyridine at room temperature for 2 h. After the usual working up, 25 mg of a ketone was isolated with the composition $C_{20}H_{24}D_6O_3$, mp 174-176°C, M^+ (main peak) 324.

Deuteration of Oxostachysolone. Oxostachysolone [2] (60 mg) was deuterated as described above for stachysolone. The yield of deuterooxostachysolone with the composition $C_{20}H_{21}D_9O_3$ (XII) was 50 mg, mp 173-175°C, M^+ (main peak) 327.

SUMMARY

It has been shown that stachysolone – a bitter substance from Stachys annua L. – is a diterpenoid with a rearranged labdane hydrocarbon skeleton of the kolavane type. Structural formula (I) has been established for stachysolone.

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