STRUCTURE OF ANNUANONE

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In Stachys annua L., family Labiatae (hedgenettle betony) we have previously found stachysolone and its acetates [1]. The result of an investigation of saponified extracts from this plant has enabled us to isolate and characterize three new auxilliary diterpenoids (Table 1).

The present paper gives a proof of the structure and stereochemistry of the chief of these compounds – the keto diol annuanone (I). On the basis of its elementary analysis and IR, mass, and NMR spectroscopy it has been concluded that the structure of annuanone is similar to that of stachysolone, which has been demonstrated [2]. It, too, is an α,β-unsaturated ketone containing trisubstituted and vinyl double bonds, and one axial secondary and one tertiary hydroxy groups.

Annuanone, just like stachysolone, is acetylated by acetic anhydride in pyridine only with heating to 60°C to form a mixture of mono- and diacetates. On oxidation with chromium trioxide it is readily converted into ketoannuanone (II), and on hydrogenation as a catalyst it gives a tetrahydro derivative (III).

In the NMR spectrum of (I) there are the signals of the same protons as in the spectrum of stachysolone. The mass spectra of these two diterpenoids are also largely similar. In the region of high mass numbers, apart from the peak of the molecular ion with m/e 320 (10%)–both have peaks corresponding to the fragments $[M-CH_3]^+$, $[M-H_2O]^+$, $[M-(CH=CH_2)]^+$, $[M-(CH_3+H_2O)]^+$ and $[M-(2H_2O+CH_3)]^+$. This confirms the common nature of the decomposition of the molecules of these substances. However, the mass spectrum of annuanone also has some differences. Thus, the peak corresponding to the fragment $[M-2H_2O]$ (m/e 284) is far stronger than the analogous peak in the spectrum of stachysolone and, conversely, the peaks relating to the residues from the successive detachment of side chains [M–70], [M–85], and [M–99] and the peaks with m/e 217 are weaker (2, 1, 35, and 5% of the maximum) as compared with the corresponding peaks in stachysolone (24, 28, 76, and 40%). The mass spectrum of annuanone differs most considerably by the fact that it shows a strong peak with m/e 219 which is practically absent from the spectrum of stachysolone.

The facts presented permit the assumption that the structure of annuanone differs from that of stachysolone by its configuration and that this is the reason for the change in the nature of the fragmentation of the side chain of the diterpenoid (I).

In the region of mass numbers below m/e 217, the spectra of annuanone and stachysolone differ only in the intensities of the individual peaks.

In the spectrum of the thioketal of tetrahydroannuanone (IV) and the corresponding derivative of tetrahydrostachysolone [2], there is the peak of a fragment (VII) with m/e 145, which confirms that the keto groups have the same position in the two diterpenoids. This is also in harmony with the displacement of the peak of the molecular ion of the deutero derivative of annuanone (by 6 amu), which is possible only if the keto group is located at C_2.

TABLE 1. Minor Diterpenoids of Stachys Annua L.

Compound	Empirical formula	mp, °C	$[\alpha]_D$, deg	M^+
Annuanone (I)	$C_{20}H_{32}O_3$	171–172	+25	320
Stachylone	$C_{20}H_{34}O_3$	140–141	+32,4	322
Stachone	$C_{20}H_{36}O_3$	146–147	–20	324

The replacement of three hydrogen atoms by deuterium in the ketothioketal (V) when it was heated with deuterium oxide in an alkaline medium shows the location of the secondary hydroxyl at C_7.

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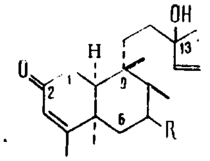
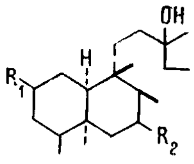
What has been said above shows that annuanone, just like stachysolone belongs to the group of diterpenoids with a rearranged labdane skeleton and has an arrangement of the functional groups similar to it (see Scheme 1).

Consequently, it may be considered that annuanone is a stereoisomer of stachysolone consisting of a diterpenoid with the trans(5α, 10β) linkage of the rings [3], all the more since their specific rotations differ in sign.

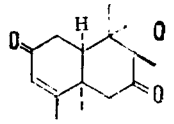
The Jones oxidation of annuanone gave a triketone (VI) not identical with the similar derivative of stachysolone. This means that the molecules of these two diterpenoids differ by the configuration of other asymmetric centers apart from C_7 and C_{13}. These may be the C_5, C_{10} or the C_9, C_8 atoms.



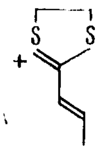
I, II



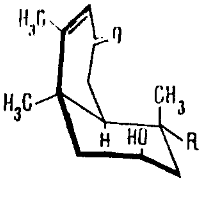
III, IV, V



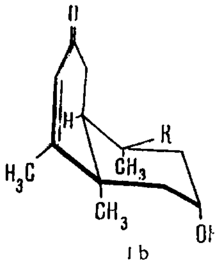
VI



VII



Ia



I b

I. R=β-OH II. R==O III. R_1==O, R_2=β-OH IV. R_1=S S, R_2=β-OH V. R_1=S S, R_2=O

The resonance signal of the C_8-methyl group in ketoannuanone (II), as in ketostachysolone [2] shifts slightly upfield (Δ = 0.08 ppm) on passing from chloroform to benzene solution. Consequently, this methyl group must be equatorially oriented in both diterpenoids [4]. The side chain at C_9 occupies the energetically most favorable equatorial position. Thus, only a difference in the linkage of rings A/B can be the main cause of the isomerism of annuanone.

A confirmation of this is formed by the optical rotatory dispersion (ORD) and circulat dichroism (CD) curves of annuanone (Fig. 1), which are very similar in the shape and sign of the Cotton effect to the curves of Δ^1-5β-3-ketosteroids [5]. The CD curve shows particularly clearly the cis (5α, 10α) linkage of rings A/B of annuanone, since it is similar to the curve of Δ^1-5-β-androst-1-en-3-one derivatives.

Further support to the facts presented concerning the cis-linkage of rings A/B of annuanone is provided by its mass spectrum, which has the above-mentioned characteristic peak with m/e 219. Its formation can be explained if it is assumed that the splitting off of the hydroxy group from the C_{13} position in the

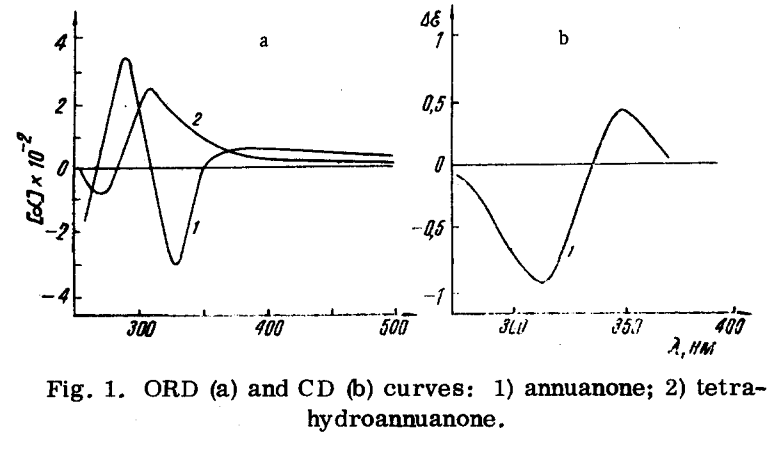
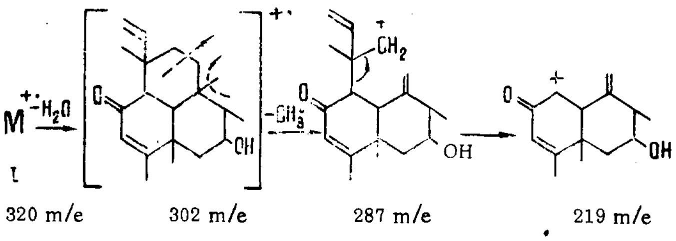


Fig. 1. ORD (a) and CD (b) curves: 1) annuanone; 2) tetrahydroannuanone.

form of water takes place with the departure of one hydrogen atom from the bicyclic system. This must be one of the six hydrogen atoms replaceable by deuterium, since the mass spectrum of deuteroannuanone shows a peak corresponding to the ejection of 19 amu (HDO) instead of 18 (H_2O) in the case of the initial substance.

A consideration of a molecular model of annuanone with the cis linkage of the rings readily shows that as a result of free rotation, the side chain can become fairly close only to the hydrogens at C_1. Then dehydration under the conditions of mass spectrometry takes place with the formation of a tricyclic ion with m/e 302, the further fragmentation of which gives a stable ion with m/e 219. As shown in the scheme below



the fragmentation of annuanone also explains the absence of peaks of $[M-70]^+$ and $[M-85]^+$, which are characteristic of stachysolone, from its spectrum. Consequently, annuanone belongs to the dicyclic diterpenoids with the cis (5α, 10α) linkage of rings A/B, and two conformations are possible for it: steroid-like (Ia) and nonsteroid (Ib). The choice between these was made according to the octant rule as applied to tetrahydroannuanone (III).

On the basis of octant diagrams [6], for the steroid-like conformation a curve was predicted with a weakly positive Cotton effect, and for the nonsteroid conformation, one with a negative effect. As can be seen from Fig. 1, the measured ORD curve of tetrahydroannuanone with a positive Cotton effect corresponds to the steroid conformation (III), and, consequently, to the same conformation for annuanone (Ia).

EXPERIMENTAL

The UV spectra were taken on a UR-10 spectrometer, the UV spectra on a Specord UV-Vis, the NMR spectra on RS-60 and Varian-A-56/60 A instruments (with TMS as internal standard), 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 evaporator temperature of 110°C and an ionizing voltage of 70 V. The ORD and CD curves were obtained on a Spectropol instrument by V. A. Babkin (Institute of Organic Chemistry, Novosibirsk). The melting points of the substances were determined on a Kofler block. The analyses of all the compounds corresponded to the calculated figures.

Isolation of the Minor Diterpenoids. An acetone extract of the plant freed from waxes and acidic products [1] (150 g) was dissolved in 600 ml of methanol containing 10% KOH. The solution was boiled for 2 h under reflux. The methanol was distilled off, the residue was treated with 200 ml of water and extracted with ether. The ethereal solution was washed to neutrality with water, dried with sodium sulfate, and distilled. The combined neutral substances obtained (24 g) were chromatographed on 1150 g of alumina (activity grade III).

Petroleum ether eluted 1.5 g of waxes, and benzene 10 g of a yellow liquid containing mainly phytol (identified by its mp, by TLC, and by IR spectroscopy with an authentic sample). A mixture of benzene and 5% of ether eluted 9.5 g of β-sitosterol. Benzene containing 15% of ether eluted 60 mg of stachone with mp 146-147°C (petroleum ether–benzene), $[\alpha]_D^{20} -20°$ (c 0.06; methanol). The IR spectrum (KBr) was identical with the spectrum of tetrahydrostachysolone [1].

A mixture of benzene with 20% of ether eluted 150 mg of crystalline stachylone, $C_{20}H_{34}O_3$, mp 140-141°C (from petroleum ether–benzene), $[\alpha]_D^{26} +32.4°$(c 1.85; chloroform). UV spectra: λ_{max} (ethanol) 204 nm (ε 710). IR spectrum (KBr), cm^{-1}: 1640, 3050 (double bond), 1700 (ketone), and 3500 (hydroxyl).

After stachylone, the same solvent eluted from the column a fraction (4.5 g) consisting mainly of two substances. When it was rechromatographed on 170 g of silica gel, a mixture of benzene with 20% of ether eluted 250 mg of annuanone, $C_{20}H_{32}O_3$, mol. wt. 320 (mass spectrum). After crystallization from methylene chloride with the addition of petroleum ether, mp 171-172°C, $[\alpha]_D^{21} +25°$ (c 8; chloroform). UV spectrum: λ_{max} (ethanol) 235 nm (ε 11,800). IR spectrum (KBr), cm^{-1}: 3440, 3480 (hydroxyl), 1655 (α,β-unsaturated ketone), 3100, 905, 860, 1610 (double bonds). NMR spectrum (pyridine): 1.15 ppm (3H, doublet, J = 6 Hz, methyl at C_8): 1.21, 1.35, 1.44, and 1.67 (singlets, 3H each, methyls at C_9, C_5, C_{13}, and C_4, respectively): 4.05 (H_7); 4.94 (1H, doublet of doublets, J_1 = 10.5, J_2 = 2.5 Hz, H_{15}); 5.28 (1H, doublet of doublets. J_1 = 17, J_2 = 2.5, $H_{15'}$); 5.60 (H_3); and 5.87 ppm (1H, triplet, H_{14}). Mass spectrum, m/e (intensity as % of the maximum peak in parentheses): 320, (10), 305 (2.5), 302 (11.5), 292 (2), 287 (6), 284 (7), 269 (6), 250 (2), 235 (1), 221 (35), 220 (7), 219 (29), 217 (5), 203 (26), 201 (12), 185 (10), 175 (20), 165 (31), 164 (27), 147 (33), 137 (31), 123 (39), 121 (30), 109 (26), 96 (35), 82 (31), 71 (60), 55 (58), 43 (100), ORD: $[\alpha]_{500}+32°$, $[\alpha]_{370}+70°$, $[\alpha]_{350}\,0°$, $[\alpha]_{350}-305°$, $[\alpha]_{315}\,0°$, $[\alpha]_{290}+387°$, $[\alpha]_{265}\,0°$, $[\alpha]_{260}-210°$ (c 0.126 methanol); CD: λ nm (Δε): 370 (+0.06), 348 (+0.41), 334 (0), 320 (−0.68), 312 (−0.98), 300 (−0.70), 278 (−0.12).

Further elution with the same solvent yielded 1.3 g of stachysolone with mp 153-155°C [1]. NMR spectrum (pyridine): 1.3 ppm (doublet, J = 6); 1.20, 1.38, 1.47, and 1.71 ppm (methyl groups); 4.02 ppm (H_7); 5.61 ppm (H_3); and 4.98, 5.28, and 5.86 ppm (vinyl protons).

Deuteration of Annuanone. Annuanone (20 mg) was dissolved in 1 ml of dry dioxane containing 0.2 ml of deuterium oxide and 10 mg of metallic sodium. The mixture was boiled for 10 min and was evaporated to dryness. The residue was treated with 0.2 ml of dioxane and 0.1 ml of deuterium oxide and the mixture was heated again. The experiment was repeated four times. The residue after final evaporation was dissolved in absolute ether, and was washed free from alkali with deuterium oxide (2 × 0.1 ml), and then with water. The ethereal solution was dried and the ether was distilled off, giving 20 mg of deuteroannuanone, $C_{20}H_{26}D_6O_3$ with mp 168-170°C, M^+ (main peak) 326.

Oxidation of Annuanone. A. To the complex obtained from 150 mg of chromium trioxide and 1.5 ml of anhydrous pyridine was added a soltuion of 80 mg of annuanone in 1.5 ml of pyridine, and the mixture was left at room temperature for 5 h. After the usual working up and chromatography (benzene containing 15% of ether) on 4 g of alumina (activity grade III), a fraction enriched in ketoannuanone was obtained. By preparative TLC on alumina, it yielded 70 mg of pure crystalline keto annuanone, $C_{20}H_{30}O_3$ (II), mol. wt. 318 (mass spectrum), mp 61°. IR spectrum ($CHCl_3$), cm^{-1}: 3610, 3500 (hydroxyl), 1710 (ketone), 1670 (α,β-unsaturated ketone), 1640, 905, 860, 3100 (double bonds).

B. The Jones reagent was added dropwise over 5 h at 0°C to 100 mg of annuanone in 5 ml of acetone, until the mixture had acquired a permanent yellow color. Then 50 ml of water were added and the mixture was extracted with chloroform. The chloroform extracts were combined, washed with 1% aqueous alkali, and with water, and dried with sodium sulfate. After distillation of the solvent and chromatography of the residue in benzene on 2 g of alumina (activity grade III), 50 mg of crystalline substance (VI) was obtained with the composition $C_{18}H_{26}O_3$, mol. wt. 290 (mass spectrum), mp 128-130°C (petroleum ether−benzene). IR spectrum (KBr), cm^{-1}: 1710 (ketone), 1665 (α,β-unsaturated ketone). A mixture with the analogous product obtained from stachysolone (mp 144-146°C) melted at 119-123°C.

Hydrogenation of Annuanone. In 10 ml of ethyl acetate in the presence of 5% $Pd/SrCO_3$, 200 mg of annuanone was saturated with hydrogen. At 26°C and 753 mm, 33 ml of hydrogen was absorbed, which corresponds to 2.13 equivalents. After the usual working up and crystallization from petroleum ether containing benzene, 150 mg of tetrahydroannuanone (III) were isolated with the composition $C_{20}H_{36}O_3$ (III), mol. wt. 324 (mass spectrum), mp 128-129.5°C, IR spectrum (KBr), cm^{-1}: 3410, 3450 (hydroxyl), 1695 (ketone); ORD: $[\alpha]_{500}+13°$, $[\alpha]_{350}+100°$, $[\alpha]_{311}+265°$, $[\alpha]_{282}\,0°$, $[\alpha]_{270}-53°$, $[\alpha]_{250}\,0°$ (c 0.241, methanol).

Thioketal of Tetrahydroannuanone (IV). A mixture of 100 mg of tetrahydroannuanone, 0.5 ml of glacial acetic acid, 100 mg of ethanedithiol, and 1 drop of borontrifluoride etherate was left at room temperature for 5 days. Then it was diluted with water, the thioketal was extracted with ether, and the ethereal extracts were washed with 5% caustic soda solution and with water, dried with sodium sulfate, and evaporated. The residue (70 mg) was chromatographed on silica gel (3.5 g). A mixture of benzene and 5% of ether eluted 50 mg of thioketal $C_{22}H_{40}S_2O_2$ (IV), mol. wt. 400 (mass spectrum), mp 164-166°C (methylene chloride−petroleum ether). IR spectrum (KBr): 3450-3500 cm^{-1} (hydroxyls).

Substance (IV) (40 mg) was oxidized with chromium trioxide in pyridine at room temperature for 6 h. After the usual working up, 30 mg of the ketothioketal (V), $C_{22}H_{38}O_2S_2$ M^+ 398 m/e, was obtained.

Compound (V) (20 mg) was deuterated as described above for annuanone. This gave 20 mg of the deuterium derivative $C_{22}H_{35}D_3S_2O_2$, M^+ (main peak) 401 m/e.

CONCLUSIONS

The structure and stereochemistry of a new diterpenoid annuanone, isolated from the plant Stachys annua L. has been shown. It has been established that it belongs to the group of compounds with a rearranged labdane carbon skeleton and with the cis (5α, 10α) linkage of rings A/B.

LITERATURE CITED

1. T. M. Orgiyan and D. P. Popa, Khim. Prirodn. Soedin., 7 (1969).
2. D. P. Popa, T. M. Orgiyan, Z. Samek, and L. Doleish, Khim. Prirodn. Soedin., 295 (1972).
3. D. P. Popa and T. M. Orgiyan, Khim. Prirodn. Soedin., 735 (1972).
4. D. H. Williams and N. S. Bhacca, Tetrahedron, 21, 2021 (1965).
5. G. Snatzke and H. W. Fehlhaber, Tetrahedron, 20, 1243 (1964).
6. W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, J. Am. Chem. Soc., 83, 4013 (1961).