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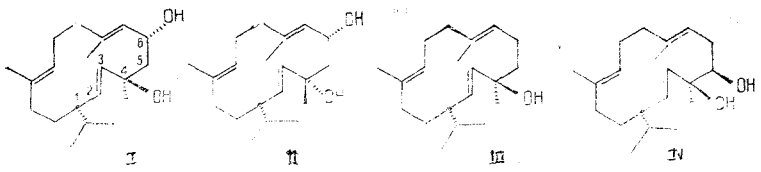
CEMBRANE ALCOHOLS — A NEW TYPE OF HORMONAL PLANT GROWTH INHIBITOR

V. A. Raldugin, V. E. Kozlov,
V. M. Chekurov, N. I. Yaroshenko,
and V. A. Pentegova

UDC 547.594.4.03

Three new cembrane alcohols have been synthesized and have been tested for growth-inhibiting activity together with a number of known compounds. By the selection and testing of cembrane derivatives a structural fragment has been found which, as is assumed, is responsible for the appearance of the growth-inhibiting activity of cembrane alcohols.

Springer et al. [1] have established that the diols (I) and (II) isolated in 1962 by Roberts and Rowland [2] from tobacco leaves are hormonal plant growth inhibitors. In view of this, the question arises of whether this biological activity is a property only of the diols (I) and (II) or is also characteristic of other cembrane compounds having common structural features. In order to investigate this, we have carried out -rials in a standard test on sections of wheat coleoptiles [3] a number of cembrane alcohols described previously and some obtained for the first time. The results are given in Table 1.



I II III IV

To establish the role of the secondary hydroxy groups in the manifestation of the biological activity of the diol (I), we tested a substance not containing this group — isocembrol (III) — and an isomer of the diol (I) — the diol (IV). It was found that the presence or absence of a secondary hydroxy group at C_6 or C_5 did not appreciably affect the growth-inhibiting activity of cembrane compounds having a hydroxy group at C_4. These substances were not inferior to diol (I) in activity.

When the tertiary hydroxy group was eliminated from the molecule of the diol (IV) (the alcohol (V)), activity appeared only at a high concentration of the solution. A similar fall in biological activity was observed for the 2,3-epoxy derivative of isocembrol (VI).

Thus, for the manifestation of hormonal growth-inhibiting activity the presence of fragment A ($R=CH_3$) in the molecule of a cembrane compound is apparently necessary.

It is interesting to note that when one of the trisubstituted double bonds of isocembrol was epoxidized (the epoxy alcohol (VII)), the activity rose, probably through the increase in the polarity of the molecule. (See scheme on following page.)

The epimerization of isocembrol at C_4 (4-epiisocembrol (VIII)) led to decrease in activity, as for the diol (II) isolated from tobacco leaves. On the other hand, replacement

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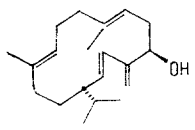
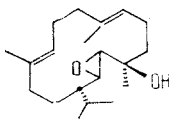
TABLE 1. Results of Tests of Compounds in Inhibition of the Growth of Sections of Wheat Coleoptiles, %

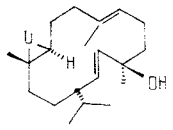
Substance	Concentration, M		
	1×10^{-3}	1×10^{-4}	1×10^{-5}
Literature figures			
Diol (I) [1]	100	60	15
Diol (II) [1]	100	60	0
Abscisic acid [1, 6]		—	73
Variety Mironovskaya 808			
Isocembrol	—	50	42
Diol (IV)		54	42
Mironovskaya Yubileinaya			
Isocembrol	75	—	24
Epoxy alcohol (VII)	100	28	27
Alcohol (X)	91.5	—	33
Krasnodarskaya 29			
Isocembrol	84	65	0
4-Epiisocembrol (VIII)	100	12	0
Alcohol (IX)	66	51	9
Alcohol (V)	50	0	0
Epoxy alcohol (VI)	23	12	0
Alcohol (XI)	70	14	0
Alcohol (XII)	73	20	0
Alcohol (XIII)	63	50	0



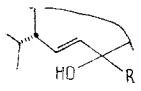
V



VI

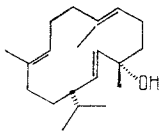


VII

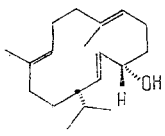


A

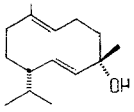
of the C_4-acyl group in 4-epiisocembrol by a hydrogen atom led to compound (IX) which was not inferior to isocembrol in activity.



VIII



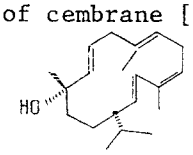
IX



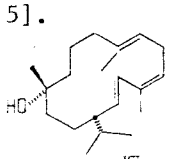
X

The next question is connected with the role of the cembrane ring in the manifestation of growth-inhibiting activity. For its elucidation we tested a lower isoprenolog of 4-epiisocembrol — the alcohol (X). It was found that this compound, in a parallel test with isocembrol, was not inferior to the latter in activity. Apparently, what is important for the manifestation of growth-inhibiting activity is the presence in the cyclic molecule of a complex of definite functional groups such as that represented by fragment A (R=H or CH_3).

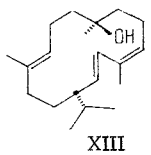
The transfer of part of fragment A (C_2-C_4) in the isocembrol molecule to another position (the alcohol (XI)) led to a decrease in the activity at a low concentration (10^{-4} M) and the same activity was retained when the C_{10} double bond in the alcohol (XI) was saturated (compound XII)). A slightly greater activity was observed for a 10^{-4} M solution of the isomeric alcohol (XIII). Apparently, alcohols (XI-XIII) are capable of being hydroxylated or hydrated *in vivo* at the C_4 double bond to active derivatives of the diol (IV) and of isocembrol. The conjugated C_4 double bond is very active in oxidation reactions, as has been shown for the case of cembrane [4, 5].



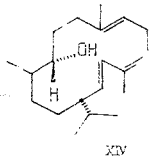
XI



XII



XIII



XIV

Thus, a growth-inhibiting effect is not a unique property of the diols (I) and (II) but is observed for other cembrane compounds containing the common structural fragment A. The size of the carbon ring is not, apparently, a decisive factor, as can be seen from the case of compound (X) and manifestation of activity can be expected for higher and lower isoprenologs and homologs of these compounds.

The cembrane inhibitors studied are inferior in activity to a well-known terpenoid hormonal inhibitor – abscisic acid [6].

A dissimilarity of the response to the compounds tested of coleoptiles from different varieties of wheat can also be observed.

The norcembrane alcohol (IX) was obtained by the reduction of the corresponding ketone [7] by lithium tetrahydroaluminate in diethyl ether. The configuration of its asymmetric center at C_4 was established as S on the basis of the observation of a positive Cotton effect at 330 nm in the circular dichroism curve of the corresponding o-nitrobenzoate [8].

Alcohols (XII) and (XIII) were obtained by the reduction of the corresponding epoxycembranes [4] with lithium tetrahydroaluminate in boiling tetrahydrofuran. The corresponding secondary alcohols were also formed with yields of from 10 to 45%, depending on the purity of the lithium tetrahydroaluminate used and on the reaction conditions. In both cases it was difficult to separate the reduction products quantitatively by chromatography on silica gel alone. More convenient for this purpose proved to be the performance of the selective acetylation of the secondary alcohols with acetic anhydride in pyridine. Their acetates were then readily separated by chromatography from the unchanged tertiary alcohols (XII) and (XIII). One of these secondary alcohols, formed in the reduction of 11,12-epoxycembrene and having structure (XIV), was characterized by its constants and spectral characteristics.

EXPERIMENTAL

Isocembrol (III) and its epimer (VIII) were isolated from the oleoresin of the Siberian stone pine [9], the alcohol (X) from the oleoresin of the Yeddo spruce [10], the diol (IV) [5] and the alcohols (V) and (XI) [11] were obtained from cembrene, and the epoxy alcohols (VI) [12] and (VII) [13] were obtained from isocembrol.

IR spectra were recorded for solutions in carbon tetrachloride on a UR-20 instrument, UV spectra for solutions in ethanol on a Specord UV-VIS instrument, and NMR spectra for solutions in carbon tetrachloride on a Varian A56/60A instrument (HMDS, δ scale) and the angles of optical rotation on a Zeiss polarimeter for solutions in chloroform.

18-Nor-4S-cembra-2E,7E,11E-trien-4-ol (IX). A solution of 0.20 g of 18-norcembra-2E, 7E,11E-trien-4-one [7] in 20 ml of absolute diethyl ether was treated with 0.1 g of lithium tetrahydroaluminate. After being stirred at room temperature for 10 min, the reaction mixture was treated with moist diethyl ether and then with water. Chromatography of the product on 15 g of silica gel gave 0.15 g of the alcohol (IX) in the form of a colorless oil with n_D^{23} 1.5090, $[\alpha]_D^{23}$ + 134.3° (c 7.15). UV spectrum: no absorption maxima in the 220-400 nm region. IR spectrum, cm^{-1}: 3620 (hydroxy group), 980 (trans-disubstituted double bond). NMR spectrum, ppm: 1.45 and 1.50 (singlets, 3 H each, methyl groups at C_8 and C_{12}), 4.03 (1 H, multiplet, width at half-height 17 Hz, H_4), 4.75-5.75 ppm (4 H, multiplet, H_2, H_3, H_7, H_{11}).

The o-nitrobenzoate of alcohol (IX) was obtained by heating a mixture of 0.1 g of the alcohol (IX) and 0.2 g of o-nitrobenzoyl chloride in 10 ml of pyridine for 10 min at 70-80°C. After the product had been purified by chromatography on silica gel, it was obtained in the form of an oil IR spectrum, cm^{-1}: 1740, 1670, 1295, 1260, 1135, 1080 (o-nitrobenzoate group), 980 (trans-disubstituted double bond).

12R-Cembra-2E,4Z,7E-trien-12-ol (XII) and the Alcohol (XIV). A solution of 0.20 g of 11S,12S-epoxycembrene [4] in 20 ml of absolute tetrahydrofuran was treated with 0.2 g of lithium tetrahydroaluminate, and the mixture was boiled under reflux for 1 h. After the usual working up, a product, (0.18 g) was obtained which consisted, according to TLC on Silufol, of two similar substances. Its chromatography on silica gel gave successively the alcohol (XIV) (cembra-2E,4Z,7E-trien-11S-ol) (0.05 g) in the form of an oil with n_D^{21} 1.5220 and $[\alpha]_D^{21}$ + 43.5° (c 6.44) and the alcohol (XII) (0.07 g) in the form of an oil with n_D^{21} 1.5160 and $[\alpha]_D^{22}$ + 78° (c 4.36), UV spectrum: λ_{max} 243 nm (log ε 4.21); IR spectrum: 3620 cm^{-1} (hydroxy group); PMR spectrum, ppm: 1.00 (3 H, singlet, C_{12}-CH_3), 1.60 and 1.76 (3 H

each, singlets, C_7-CH_3 and C_4-CH_3, respectively), 2.67 (2 H, triplet of multiplets with J ~ 7 Hz, protons at C_6), and 5.00 and 5.50 ppm (1 H each, triplets of multiplets with J ~ 7 Hz, H_7 and H_5, respectively). The H_2 and H_3 protons form an AB system the components of which are present in the spectrum at 5.21, 5.33, 5.48, 5.60 (H_2), 6.00 and 6.27 ppm (H_3), $J_{2,3}$ = 16 Hz (the H_2 proton also interacts with H_1; $J_{1,2}$ = 7 Hz).

The NMR spectrum of the alcohol (XIV) exhibits a multiplet at 0.8 ppm (9 H, methyls of an isopropyl group and C_{12}-CH_3), singlets at 1.56 and 1.67 ppm (methyl groups at C_7 and C_4, respectively), and a multiplet at 3.26 ppm (1 H, proton at a secondary hydroxy group). The signals of the olefinic protons are similar to those observed in the NMR spectrum of the alcohol (XII). In the NMR spectrum of the acetate of the alcohol (XIII), the signal of the proton at the acetoxy group (C_{11}-H) is observed at 4.40 ppm and the protons of the acetoxy group itself give a singlet at 1.81 ppm. The remainder of the NMR spectrum of the acetate is similar to that of the initial alcohol.

The ratio of the alcohols (XII) and (XIV) formed in this experiment was 55:45, which was determined from the integral intensities of the signals of the H_3 proton; they were doublets with $J_{2,3}$ = 16 Hz in both cases but differ in the values of their chemical shifts by 0.1 ppm.

Cembra-2E,4Z,11E-trien-8S-ol (XIII). Alcohol (XIII) was obtained by the reduction of 7S,8S-epoxycembrene [4] by a method analogous to that described above for the synthesis of the alcohol (XII). The product, likewise consisting, according to TLC, of two similar compounds, was acetylated with acetic anhydride in pyridine (50-60°C, 2 h), and then, by chromatography on silica gel, the unchanged fraction — the alcohol (XIII) (yield 60%) — and the acetate of the isomeric alcohol — cembra-2E,4Z,11E-trien-7S-ol (yield 30%) — were isolated by chromatography on silica gel.

The alcohol (XIII) had the form of a colorless oil with n_D^{23} 1.5112 and $[\alpha]_D^{21}$+ 14.5° (c 6.90), UV spectrum: λ_{max} 242 nm (log ε 3.59). IR spectrum, cm^{-1}: 3620 (hydroxy group), 980 (trans-disubstituted double bond). NMR spectrum (ppm) 1.02, 1.46, and 1.74 (3 H each, singlets, methyl groups at C_8, C_{12}, and C_4, respectively), 5.03-5.55 (3 H, multiplet, H_2, H_5, and H_{11}). The signal of the H_3 proton has the form of a distorted doublet ($J_{2,3}$ = 16 Hz) with components at 5.16 and 5.43 ppm.

The acetate of cembra-2E,4Z,11E-trien-7S-ol was obtained in the form of a colorless oil. IR spectrum, cm^{-1}: 980 (trans-disubstituted double bond), 1730 (carbonyl of an acetoxy group), and the NMR spectrum was similar to that of the acetate of the alcohol (XIV).

Performance of the Tests for Inhibiting Activity. For the tests, wheat seed sprouts (coleoptiles) 18-22 mm long were selected. The tip (5 mm) was cut off from each coleoptile and the next 5 mm of the coleoptile was used in the experiment. For each concentration of substance under investigation three test tubes (replicates) were set up. A sample of the substance in the test tube was dissolved in 0.03 ml of ethanol, and 2 ml of nutrient medium (2 g of sucrose and 1.5 mg of indolylacetic acid in 100 ml of phosphate buffer with pH 5.0) was added. Then, ten coleoptiles were introduced into the test tube. The tubes were placed in a rotating drum in a thermostat at +26°C for 20 h. After this, the lengths of the sections of the coleoptiles were measured with an accuracy of 1 mm. Control tubes each contained 0.03 ml of ethanol, 2 ml of nutrient medium, and 10 coleoptile sections. The mean length of the control shoots after incubation was taken as 100%.

The degree of inhibition is the difference between the mean lengths of the control and experimental coleoptile sections expressed as a percentage of the mean length of the control coleoptile sections (see Table 1).

SUMMARY

1. Growth-inhibiting activity is not a property of the two diols isolated from tobacco leaves, alone but is also characteristic for other related, cembrane alcohols.

2. The degree of expression of growth-inhibiting activity in a series of cembrane alcohols depends on the functional groups present in their molecules and is a maximum for compounds structurally similar to isocembrol.

3. Four new cembrane alcohols have been synthesized and three of them have been tested for growth-inhibiting activity.

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PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS *Silene*.

III. SILENEOSIDE A — A NEW GLYCOSIDIC ECDYSTEROID OF *Silene brachuica*

Z. Saatov, M. B. Gorovits,
N. D. Abdullaev, B. Z. Yasmanov,
and N. K. Abubakirov

UDC 547.927;591.147

Ecdysterone (I), viticosterone E, polypodine B, and integristerone A (II) have been isolated from the epigeal part of the plant *Silene brachuica* Boiss. In addition to substances (I) and (II), the phytoecdysteroid sileneoside A has been isolated from the root of this plant. It has been shown that sileneoside A is ecdysterone 22-O-α-D-galactoside.

We are continuing a study of the ecdysteroids of plants of the genus *Silene* (family Caryophyllaceae) [1]. The presence of ecdysterone (I), viticosterone E (II), polypodine B (III), and integristerone A (IV) in the epigeal parts of the *Silene brachuica* Boiss. has been shown. Five ecdysteroids have been detected in the roots of the plant, and these have been denoted in order of increasing polarity as substances A, B, C, D, and E. Components A and C have been identified, respectively, as ecdysterone (I) and integristerone A (IV). The other phytoecdysteroids have proved to be new. In the present communication the structure of product B, which we have called sileneoside A (V) is considered.

In the UV spectrum of compound (V), the α,β-unsaturated ketone grouping that is characteristic for the ecdysteroids is revealed by a maximum at 246 nm (log ε 4.15), and in the IR spectrum it is shown by absorption at 1645 cm^{-1}. The positions of the maxima and the size of the dichromic absorption [$\Delta\varepsilon = -5.03$ (249 nm); $\Delta\varepsilon = 2.01$ (330 nm)] of the CD curve of compound (V) are indicative for 5β-ecdysteroids [2].

The presence in the mass spectrum of substance (V) of the products of the successive dehydration of the molecular ion with m/z 624, 606, 588, and 570, in combination with fragments having m/z 363, 345, 327, 99, 81, and 69, characteristic of ecdysteroids [3, 4], and

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