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WITHANOLIDES – A NEW TYPE OF PHYTOSTEROIDS

A. V. Kamernitskii, I. G. Reshetova, and V. A. Krivoruchko

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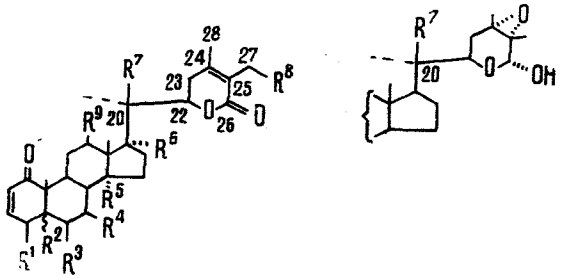
In recent years, to the types of steroids known previously [1, 2] a series of new ones isolated from natural sources of animal and vegetable origin has been added.

Some of the newly discovered types of steroid compounds are of great interest for their biological properties. Thus, compounds possessing a steroid skeleton of the ergostane type with a pyran ring in the side chain form a single biogenetic group which is found in plants of the family Solanaceae. New phytosteroids (about 60 compounds) have been isolated from Acnistus, Datura, Dunalia, Jaborosa, Withania, and Nicandra ph. and, accordingly, they have acquire the names of withanolides, jaborosalactones, withaphysalins, and nicandrins.



Biological Activity of the Withanolides

The enormous interest shown in the new class of phytosteroids, in the determination of their structure, and in the development of schemes of synthesis has been due to their biological activity. After the first report

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[3] on the anticancer activity of extracts of the leaves of Acnistus arborescens, the intensive investigations of Withania somnifera Dun. carried out simultaneously by groups under Levie, Adam, and Snatzke in various countries led to the isolation of the active principal of these extracts – withaferin A (4). The subsequent detailed study of the activity of withaferin A showed that in extremely low concentrations of 0.01-0.5% in water it inhibits the growth of plant cancer cells [4]. In an investigation of its influence on Ehrlich ascites cancer cells [5] implanted into mice, it was found that withaferin A causes the suppression of their growth accompanied by their complete disappearance in 80% of the mice. It is not known which structural features are responsible for this effect and it is possible that in the anticancer activity of the withanolides an important role may be played by structural elements both in rings A/B and in the side chains.

The present review is devoted to a consideration of features of substitution in the steroid skeleton and the determination of the structures of the side chains, and also to approaches to the partial synthesis of these compounds. Questions of the presence of representatives of this type in nature and methods for their detection and isolation have been considered in the preceding paper of this issue [6].

At the present time, about 60 representatives of the type of steroids described have been isolated; their structures are given in Table 1.

The steroid nature of the skeleton of the group of compounds under consideration is ascribed to them on the basis of experiments [7, 8] on dehydration with SeO_2, which led to derivatives of cyclopentanophenanthrene and trimethylnaphthalene. All the withanolides, jaborosalactones, and nicandrins, with the exception of compounds (15) and (17) are characterized by a Δ^2-1-oxo grouping in ring A for which absorption at 225 nm in the UV spectrum, bands at 1690 and 1660 cm^{-1} in the IR spectrum, and weak-field signals from the C-2 and C-3 vinyl protons at 6.18 and 6.97 ppm in the PMR spectrum (Table 2) are characteristic. A consideration of the supplementary structural features in rings A/B enables these phytosteroids to be divided into a number of subgroups.

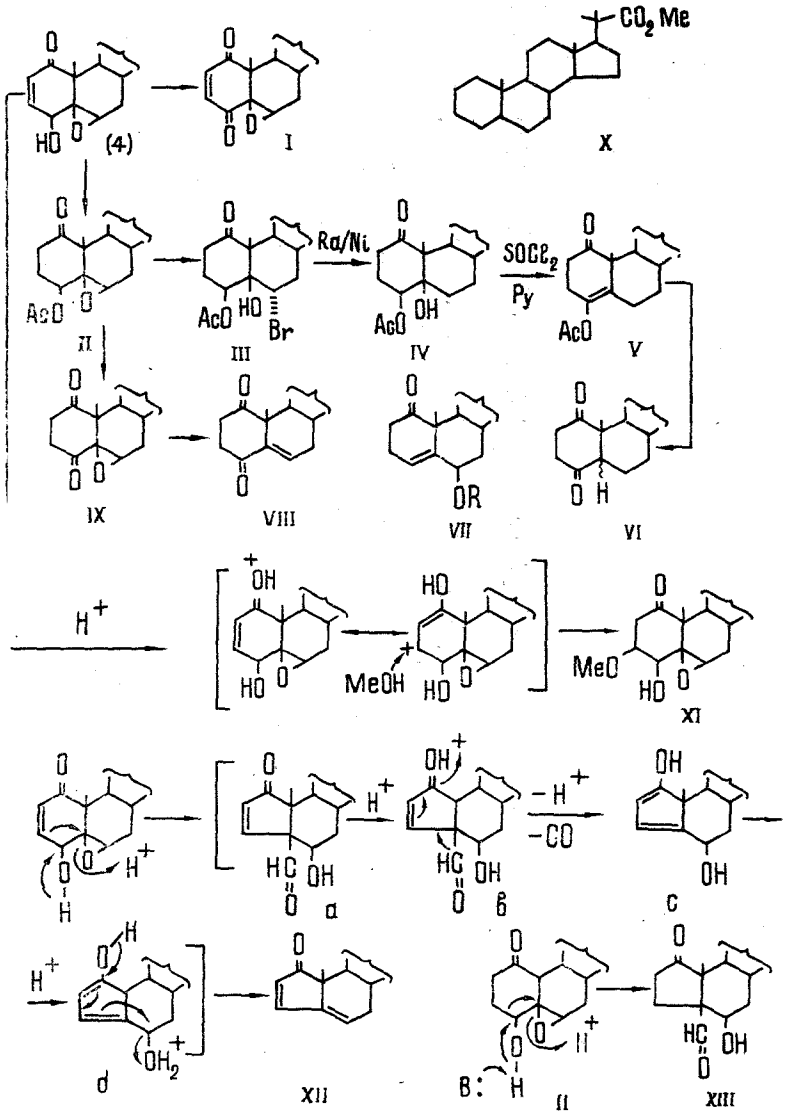
Characteristics of the Substitutions in the Steroid Skeleton

Of the 60 new phytosteroids described, 14 contain a 4β-hydroxy-5β,6β-epoxy grouping in addition to the Δ^2-1-oxo system and are characterized by the corresponding signals in the PMR spectrum (see Table 2 for compounds 1-12) [7-14]. The allyl position of the 4-OH group is confirmed by oxidation to the enedione (I) [7], having dihydroquinoid structure which can be present only in ring A of the steroid skeleton. The presence of an oxide ring in the 5,6 position is also shown by chemical transformations of the Δ^2-hydrogenated system, leading either to the opening of the epoxide or to its reduction. The identities of all the reaction products have been shown strictly by the PMR method. The conversion of (VI) into the dithioketal and its reduction followed by ozonization of the lactone group in the side chain – a 12-stage degradation of withaferin A (4) [1] to the known bisnor-(5α)-cholanic acid (X) has served as a proof of the stereochemistry of the linkage of rings A, B, C, and D. This linkage of rings A/B is also shown by the positive sign of the Cotton effect in the CD spectra at 340 nm in the region of the $n \rightarrow \pi^*$ transition of the α,β-unsaturated ketone. It has been shown by transformations of 1,4-diketo systems isomeric with respect to the C-5 center [15] that the 60 group at C-1 in Δ^2-steroids makes the cis (5β) A/B ring system more stable than the trans (5α) system, which is explained by a decrease of the nonbound interaction in the 5β series between the 1-keto group and the 11α proton.

It must be particularly noted that in a study of the transformations of the Δ^2-1-oxo-4β-hydroxy-5β,6β-epoxide system interesting reactions have been found. Thus, on treatment with p-TsOH in CH_3OH nucleophilic addition of the solvent takes place at C-3 with the formation of 3-methoxy-2,3-dihydrowithaferin A (XI) [7], or a rearrangement of the pinacolone type is observed with the formation from withaferin A of the A-nor-2,5-dien-1-one (XII) and from dihydrowithaferin of the A-nor-5-formyl derivative (XIII) [12] (see Scheme on following page.)

Withanolides having no 5,6-oxide ring, of types (32 and 33), are distinguished by the absence of a signal at 3-4 ppm in the PMR spectra, but in the weak-field region they have the signal of a vinyl proton in the form of a doublet (in 33, δ 5.78 ppm) or a multiplet (in 32, δ 6.00 ppm). A reflection of the 7-OH group in (33) is a one-proton doublet from 7-H at δ 3.84 ppm.

A second large group consists of withanolides with a polyenic system in rings A/B (21, 22, 29, 30, 34, 35) [9, 16, 17], in rings A, B, and C – $\Delta^{2,5,8(14)}$-compounds (14, 16, 18) [18] –, or in rings A, B, and D – $\Delta^{2,4,14(15)}$-compounds (19, 31) [10, 18]. They are characterized by signals from the vinyl protons in the weak-field region of the PMR spectrum (see Table 2). The position of the $\Delta^{8(14)}$ and $\Delta^{14(15)}$ bonds affects the downfield shift of the signal from the 18-CH_3 group (δ 1.03 ppm in 16, 19, and 31 as compared with, for example, 0.7 ppm in 4), while in the case of a $\Delta^{14(15)}$ bond there is a signal from the 15-H at δ 5.25 ppm. The positions of the double bonds in the compounds mentioned have been shown by chemical transformations into saturated systems or epoxides.



The nature of the multiplicity and the positions of the signals in the weak-field region varies on passing from Δ^2 compounds to $\Delta^{3,5}$-withanolides (15, 17). To these are assigned signals from the vinyl protons at C-3 and C-4 in the form of a doublet of triplets at δ 5.62 and 6.08 ppm with a coupling constant of 10 Hz [18]. The $\Delta^{3,5}$-heteroannular diene shows absorption in the UV spectrum at λ_{max} 232 nm, ε 24,000.

A considerable number of withanolides and nicandrins is characterized by 5α-hydroxy-Δ^2-1-oxo-6α,7α-epoxy grouping (23-27, 42-47) [9, 17, 19-27]. Of the three possible positions for a disubstituted oxide ring in a 17-substituted steroid Δ^2-1-ketone – 11,12;15,16; and 6,7 – the last was selected on the basis of a comparison of the products of the degradation of the side chain. The α orientation of the 5-hydroxy group was assigned on the basis of the coincidence of the change in the chemical shift from the 19-CH_3 group as a function of the nature of the solvent ($\Delta\delta \frac{CDCl_3}{C_6H_5}$ = + 0.26 ppm) and literature information for steroid 5α-1-ketones (+0.25 ppm). For the 5β isomers, this difference amounts to −0.12 ppm. The 5α stereochemistry is also confirmed by the value of $\Delta\delta \frac{CDCl_3}{C_5H_5N}$ = 0.07 and the pronounced negative sign of the Cotton effect in the region of the n → π* transition for enones at 338 nm in the CD spectrum. The α orientation of the 6,7-epoxide ring is shown, on the one hand by its preferential formation from the corresponding Δ^6 compounds and, on the other hand, by the direction of its opening by HBr-acid. From the preferential formation of the trans-diequatorial bromohydrin (XV) the influence of a neighboring 5α-OH group was deduced. The trans-diaxial bromohydrin (XVI) obtained from (42) readily undergoes ring closure at the 5α-OH group to form the 5α,6α-epoxide (XVII). An x-ray structural analysis of the nicandrins [20, 29] has shown that ring A has a half-chair conformation [C(5) below and C(2) above the plane of the ring] and ring B a half-chair conformation with C(9) below and C(10) above the plane of

TABLE 1. Compounds Isolated from Natural Sources

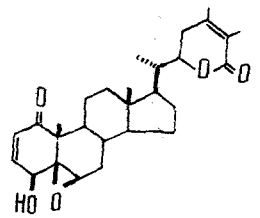
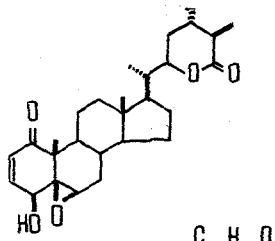
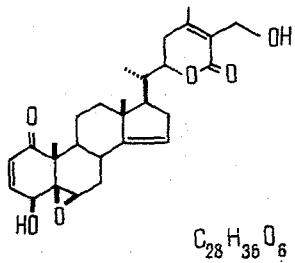
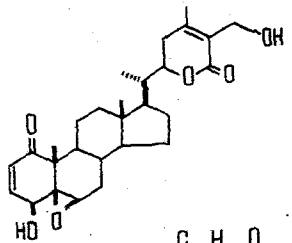
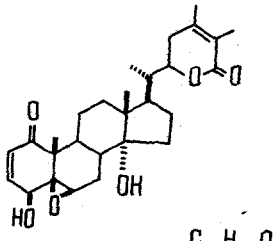
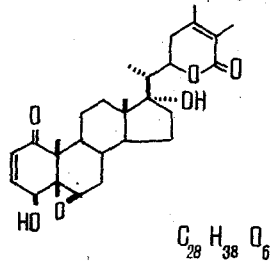
Structure and composition of the compound	Name of the compound	Source	Literature
1  $C_{28}H_{38}O_5$	27-Deoxywithaferin A (4β-hydroxy-1-oxo-5β,6β-epoxy-22R-witha-2,24-dienolide)	Withania somnifera	9,33,34
2  $C_{28}H_{40}O_5$	(4β-Hydroxy-1-oxo-5β,6β-epoxy-24,25-dihydro-22R-with-2-enolide)	Withania somnifera	33,34
3  $C_{28}H_{36}O_6$	(4β-27-Dihydroxy-1-oxo-5β,6β-epoxy-22R-witha-2,14,24-trienolide)	Withania somnifera, Withania aristata	9
4  $C_{28}H_{38}O_6$	Withaferin A (4β,27-dihydroxy-1-oxo-5β,6β-epoxy-22R-witha-2,24-dienolide)	Withania somnifera, Withania aristata, Acnistus arborescens	3,7—10, 33,47
5  $C_{28}H_{38}O_6$	14-Hydroxy-27-deoxywithaferin A (4β-14α-dihydroxy-1-oxo-5β,6β-epoxy-22R-witha-2,24-dienolide)	Withania somnifera	9,32
6  $C_{28}H_{38}O_6$	(4β,17α-Dihydroxy-1-oxo-5β,6β-epoxy-22R-witha-2,24-dienolide)	Withania somnifera	9

TABLE 1 (cont.)

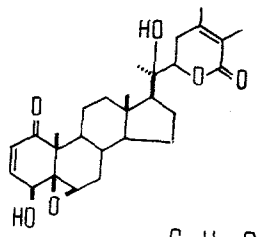
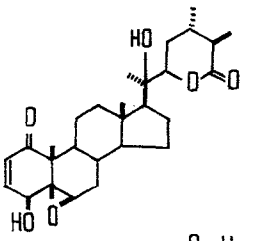
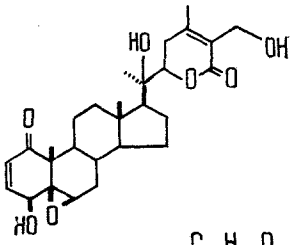
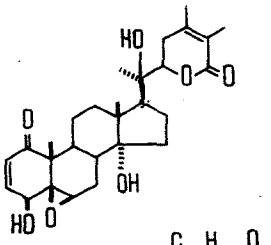
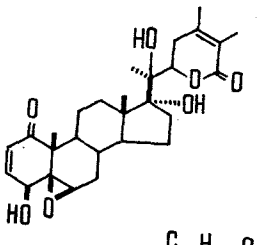
Structure and composition of the compound	Name of the compound	Source	Literature
7  $C_{28}H_{38}O_6$	Withanolide D (4β,20α-dihydroxy-1-oxo-5β,6β-epoxy-22R-witha-2,24-dienolide)	Withania somnifera	10, 33, 34
8  $C_{28}H_{40}O_6$	(4β,20α-Dihydroxy-1-oxo-5β,6β-epoxy-25-dihydro-22R-with-2-enolide)	Withania somnifera	33, 34
9  $C_{28}H_{38}O_7$	(4β,20α,27-Trihydroxy-1-oxo-5β,6β-epoxy-22R-witha-2,24-dienolide)	Withania somnifera	10
10  $C_{28}H_{38}O_7$	(4β,14α,20α-Trihydroxy-1-oxo-5β,6β-epoxy-20R,22R-witha-2,24-dienolide	Withania somnifera	10
11  $C_{28}H_{38}O_7$	(4β,17α,20α-Trihydroxy-1-oxo-5β,6β-epoxy-20S,22R-witha-2,24-dienolide)	Withania somnifera	10

TABLE 1 (cont.)

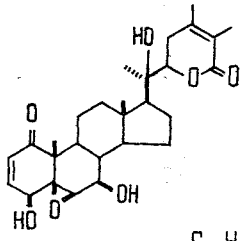
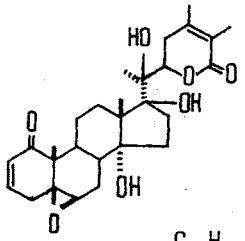
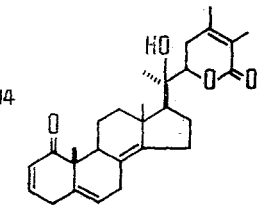
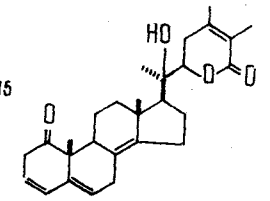
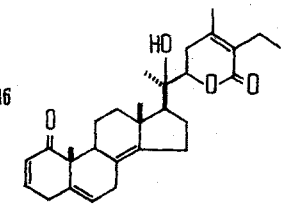
Structure and composition of the compound	Name of the compound	Source	Literature
12  $C_{28}H_{38}O_7$	(4β, 7β, 20α-Trihydroxy-1-oxo-5β, 6β-epoxy-22R-witha-2,24-dienolide)	Acnistus australis, Dunalia australis	35—37
13  $C_{28}H_{38}O_7$	Withanolide E (14α, 17β, 20-trihydroxy-1-oxo-5β, 6β-epoxy-17α-witha-2,24-dienolide)	Withania somnifera	11,18
14  $C_{28}H_{36}O_4$	Withanolide G (20α-hydroxy-1-oxo-20R, 22R-witha-2,5,8(14)-24-tretraenolide)	Withania somnifera	18
15  $C_{28}H_{36}O_4$	Withanolide I (20α-hydroxy-1-oxo-20R, 22R-witha-3,5,8(14), 24-tetraenolide)	Withania somnifera	18
16  $C_{28}H_{36}O_5$	Withanolide H (20α, 27-dihydroxy-1-oxo-20R, 22R-witha-2,5,8(14), 24-tetraenolide)	Withania somnifera	18

TABLE 1 (cont.)

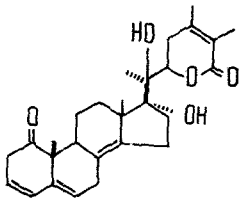
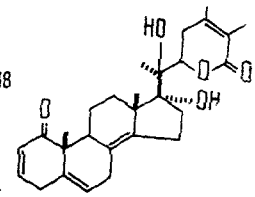
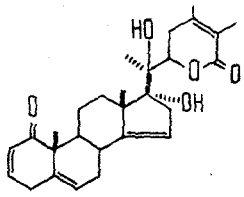
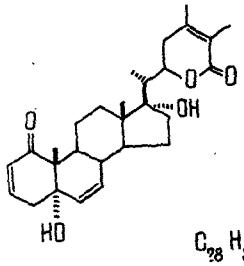
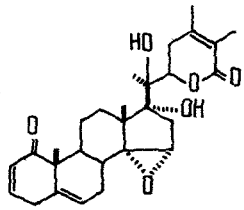
Structure and composition of the compound	Name of the compound	Source	Literature
17  $C_{28}H_{36}O_5$	Withanolide K (17α, 20α-dihydroxy-1-oxo-20R, 22R-witha-3,5,8(14), 24-tetraenolide)	**Withania somnifera**	18
18  $C_{28}H_{36}O_5$	Withanolide J (17α, 20α-dihydroxy-1-oxo-20R, 22R-witha-2,5,8(14), 24-tetraenolide)	**Withania somnifera**	18
19  $C_{28}H_{36}O_5$	Withanolide L (17α, 20α-dihydroxy-1-oxo-20R, 22R-witha-2,5,14,24-tetraenolide)	**Withania somnifera**	18
20  $C_{28}H_{38}O_5$	(5α, 17α-Dihydroxy-1-oxo-22R-witha-2,6, 24-trienolide)	**Withania somnifera**	9
21  $C_{28}H_{36}O_5$	Withanolide M (17α, 20-dihydroxy-1-oxo-14α 15α-epoxy-20R, 22R-witha-2,5, 24-trienolide)	**Withania somnifera**	18

TABLE 1 (cont.)

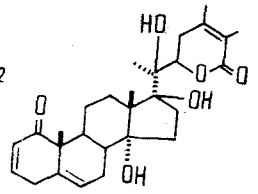
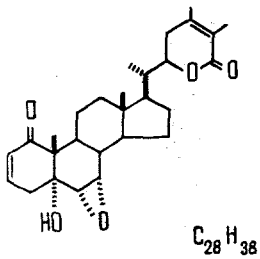
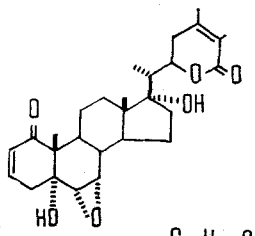
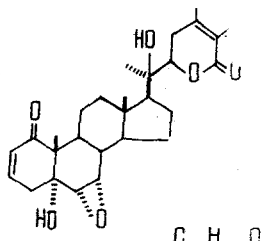
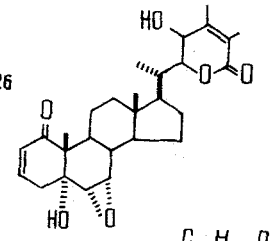
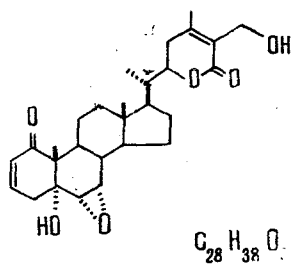
Structure and composition of the compound	Name of the compound	Source	Literature
22  $C_{28}H_{38}O_6$	Withanolide F (14α, 17β, 20-trihydroxy-1-oxo 17α-witha-2,5, 24-trienolide)	Withania somnifera	18
23  $C_{28}H_{38}O_5$	(5α-Hydroxy-1-oxo-6α, 7α-epoxy-22R-witha-2, 24-dienolide)	Lycium chinense	24
24  $C_{28}H_{38}O_6$	(5α, 17α-Dihydroxy-1-oxo-6α, 7α-epoxy-22R-witha-2,24-dienolide)	Withania somnifera	9
25  $C_{28}H_{38}O_6$	(5α, 20-Dihydroxy-1-oxo-6α, 7α-epoxy-22R-witha-2,24-dienolide)	Withania somnifera, Withania coagulans, Lycium chinense	19, 24, 25
26  $C_{28}H_{36}O_6$	Withanolide R (5α, 23-dihydroxy-1-oxo-6α, 7α-epoxy-22S, 23R-witha-2,24-dienolide)	Withania somnifera	17
27  $C_{28}H_{38}O$	(5α, 27-Dihydroxy-1-oxo-6α, 7α-epoxywitha-2, 24-dienolide)	Withania somnifera	

TABLE 1 (cont.)

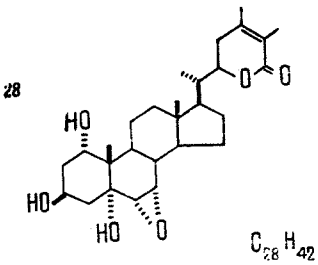
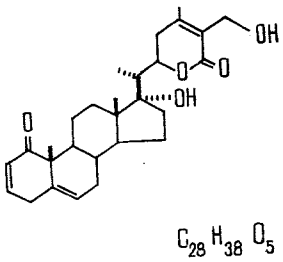
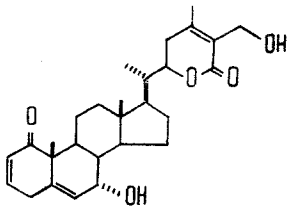
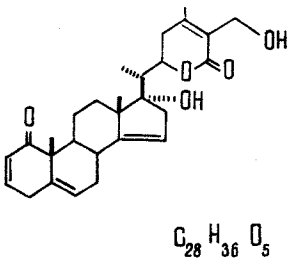
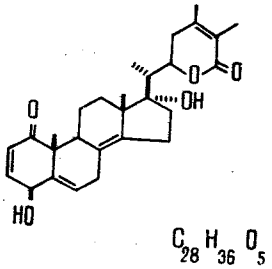
Structure and composition of the compound	Name of the compound	Source	Literature
28  $C_{28}H_{42}O_6$	(1*a*, 3*β*, 5*a*-Trihydroxy-6*a*, 7*a*-epoxy-22R-witha-24-enolide)	Withania somnifera	9
29  $C_{28}H_{38}O_5$	(17*a*, 27-Dihydroxy-1-oxo-witha-2,5,24-trienolide)	Withania somnifera	9
30  $C_{28}H_{38}O_5$	(7*a*, 27-Dihydroxy-1-oxo-witha-2,5,24-trienolide)	Withania somnifera	9
31  $C_{28}H_{36}O_5$	Withanolide N (17*a*, 27-dihydroxy-1-oxo-witha-2,5,14,24-tetraenolide)	Withania somnifera	10
32  $C_{28}H_{36}O_5$	(Withanolide O (4*β*, 17*a*-dihydroxy-1-oxo-witha-2,5,8 (14), 24-tetraenolide)	Withania somnifera	10

TABLE 1 (cont.)

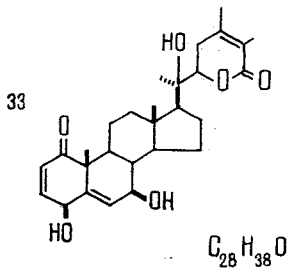
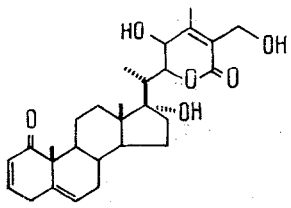
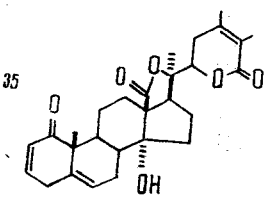
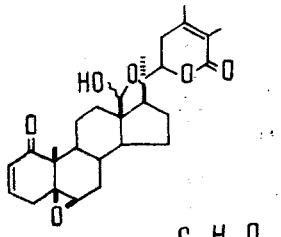
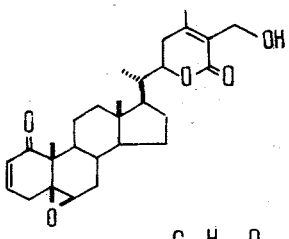
Structure and composition of the compound	Name of the compound	Source	Literature
33  $C_{28}H_{38}O_6$	(4β, 7β, 20-Trihydroxy-1-oxo-20R, 22R-witha-2,5, 24-trienolide)	Acnistus australis, -Dunalia australis	35—37
34  $C_{28}H_{38}O_6$	Withanolide Q (17α, 23,27-trihydroxy-1-oxo-22S, 22R-witha-2,5,24-trienolide)	Withania somnifera	17
35  $C_{28}H_{34}O_6$	Withaphysalin A	Physalis minima	39
36  $C_{28}H_{36}O_6$	Withaphysalin B	Physalis minima	39
37  $C_{28}H_{38}O_5$	Jaborosalactone A (4-deoxywithaferin A)	Jaborosa integrifolia	16,30

TABLE 1. (cont.)

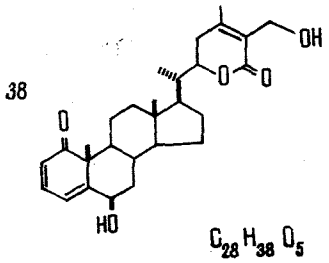
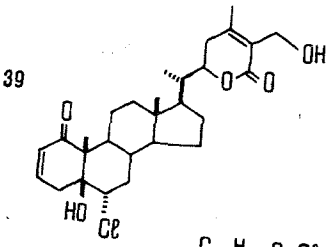
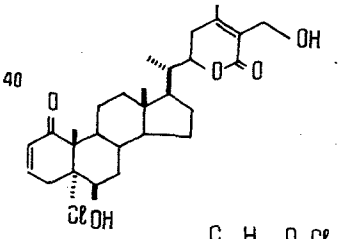
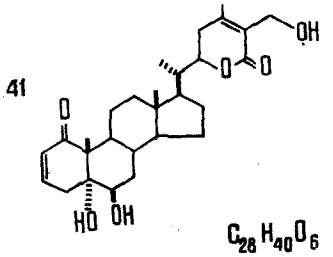
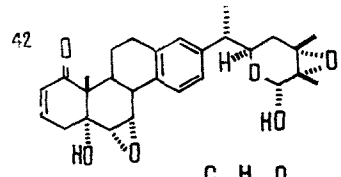
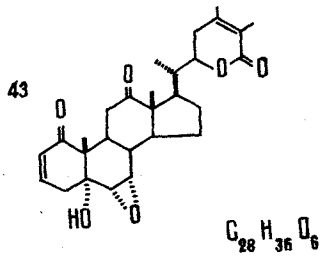
Structure and composition of the compound	Name of the compound	Source	Literature
38  $C_{28}H_{38}O_5$	Jaborosalactone B	Jaborosa integrifolia	16,30
39  $C_{28}H_{39}O_5Cl$	Jaborosalactone C	Jaborosa integrifolia	16,30
40  $C_{28}H_{39}O_5Cl$	Jaborosalactone E	Jaborosa integrifolia	16,30
41  $C_{28}H_{40}O_6$	Jaborosalactone D	Jaborosa integrifolia	16,30
42  $C_{28}H_{34}O_6$	Nicandrenone (Nic-1) (5,26-dihydroxy-6α, 7α: 22, 26: 24, 25-triepoxy-17 (13→18)-abeo-5α-ergosta-2,13, 15,16-tetraen-1-one	Nicandra physaloides	23,28,29,41
43  $C_{28}H_{36}O_6$	Withanicandrin (5α-hydroxy-1, 12-dioxo-6α, 7α-epoxy-22R-witha-2,24-dienolide)	Nicandra physaloides	21

TABLE 1 (cont.)

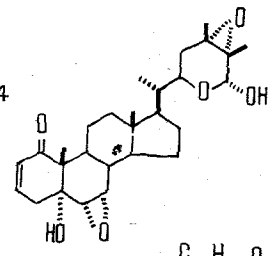
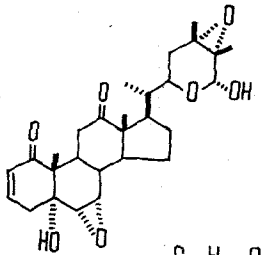
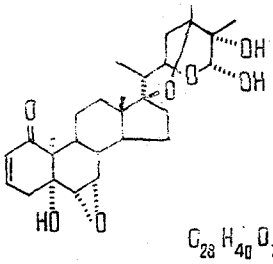
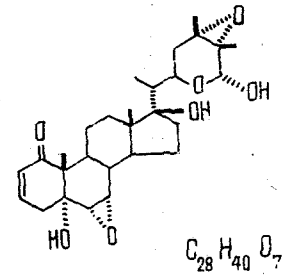
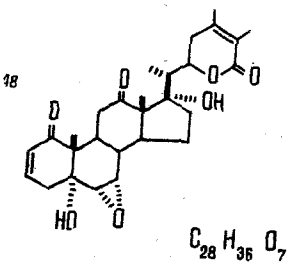
Structure and composition of the compound	Name of the compound	Source	Literature
44  $C_{28}H_{40}O_6$	Nic-3 (20S,22R, 24S, 25S, 26R-5, 26-dihydroxy-6*a*, 7*a*; 22,26; 24,25-triepoxy-5*a*-ergost-2-en-1-one)	Nicandra physaloides	20, 22
45  $C_{28}H_{38}O_7$	Nic-7 (20S, 22R, 24S, 25S, 26R-5,26-dihydroxy-6*a*, 7*a*; 22, 26; 24, 25-triepoxy-5*a*-ergost-2-ene-1,12-dione)	Nicandra physaloides	20,22
46  $C_{28}H_{40}O_7$	Nic-11 (5,25,26-Trihydroxy-6*a*, 7*a*: 17,24: 22,26-triepoxy-5*a*, 17*a*-ergost-2-en-1-one)	Nicandra physaloides	20,22
47  $C_{28}H_{40}O_7$	Nic-2 (5,17β, 26-trihydroxy-6*a*, 7*a*: 22, 26; 24, 25-triepoxy-5*a*, 17*a*-ergost-2-en--1-one)	Nicandra physaloides	22
48  $C_{28}H_{36}O_7$	(5*a*, 17*a*-Dihydroxy-1,12-dioxo-6*a*, 7*a*-epoxy-22S-witha-2,24-dienolide)	Datura quercifolia	38

TABLE 1 (cont.)

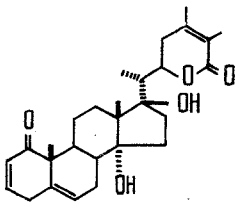
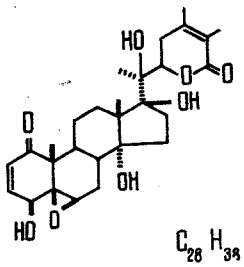
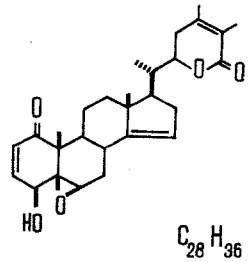
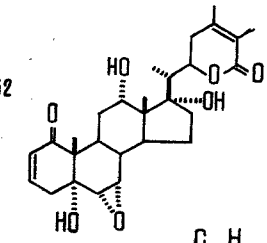
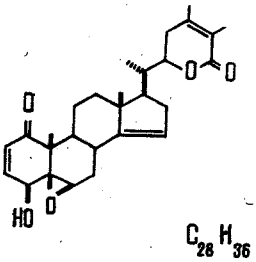
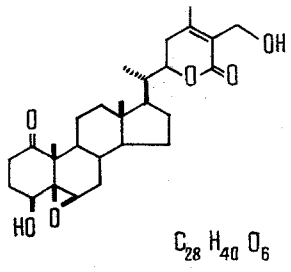
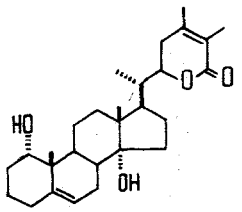
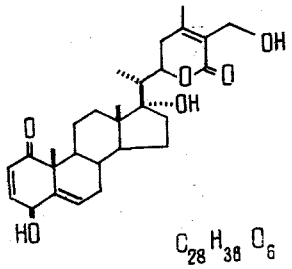
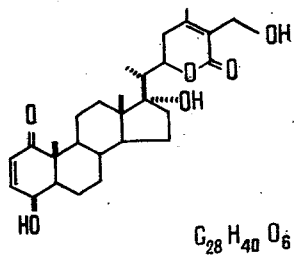
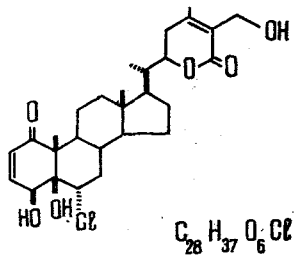
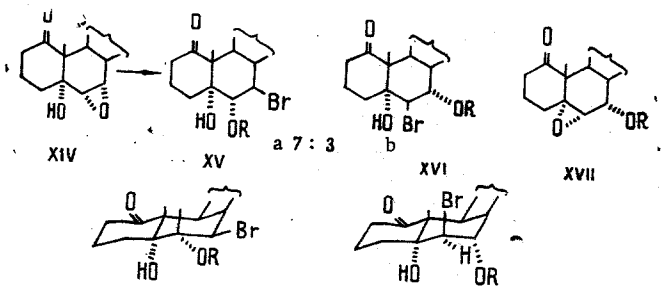
Structure and composition of the compound	Name of the compound	Source	Literature
49  $C_{28}H_{38}O_5$	Withanolide P (14α, 17β-dihydroxy-1-oxo-17α-witha-2,5,24-trienolide)	Withania somnifera	10
50  $C_{28}H_{38}O_6$	4β-Hydroxywithanolide E (4β, 14α 17β, 20-tetrahydroxy-1-oxo-5β, 6β-epoxy-17α-witha-2,24- dienolide)	Physalis peruviana	40
51  $C_{28}H_{36}O_5$	(4β-Hydroxy-1-oxo-5β, 6β-epoxy-22R-witha-2,14, -24-trienolide)	Withania somnifera	9
52  $C_{28}H_{38}O_7$	Daturalactone (5α, 12α, 17α-trihydroxy-1-oxo-6α, 7α-epoxy-22S-witha-2,24-dienolide)	Datura quercifolia	47
53  $C_{28}H_{36}O_5$	(4β-Hydroxy-1-oxo-5β, 6β-epoxy-22R-witha-2, 14, 24-trienolide)	Withania aristata	48

TABLE 1. (cont.)

Structure and composition of the compound	Name of the compound	Source	Literature
54  $C_{28}H_{40}O_6$	2,3-Dihydrowithaferin A	Withania aristata	48
55  $C_{28}H_{42}O_4$	(1α, 14α-Dihydroxy-22R-witha-5,24-dienolide)	Withania aristata	48
56  $C_{28}H_{38}O_6$	(4β, 17α, 27-Dihydroxy-1-oxo-22R-witha-2, 5, 24-trienolide)	Withania frutescens	49
57  $C_{28}H_{40}O_6$	(4β, 17α, 27-Trihydroxy-1-oxo-22R-witha-2,24-dienolide)	Withania frutescens	49
58  $C_{28}H_{37}O_6Cl$	(6α-Chloro-4β, 5β-27-trihydroxy-1-oxo-22R-witha-2,24-dienolide)	Withania frutescens	49

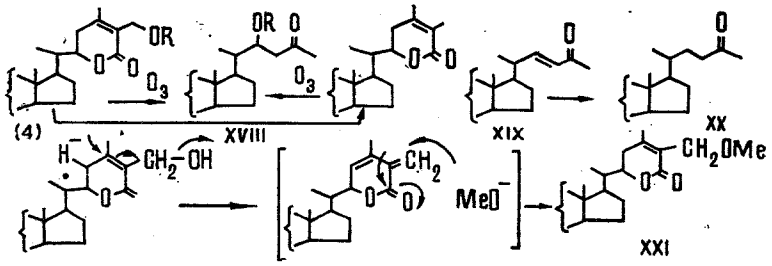
the ring. The 5α-hydroxy group does not form hydrogen bonds. The presence of a 12-keto function in withanicandrin (43) is shown by the difference of the 18-CH_3 chemical shifts, amounting to +0.38 ppm (compare δ 0.85 ppm in 24 and 25, and δ 1.11 ppm in 43) (see Scheme on following page.)

The most interesting aspect of the compounds under consideration is the structure of their side chain in position 17. A common fragment of the withanolides, jaborosalactones, and withaphysalins is a six-membered α, β-unsaturated lactone, while the nicandrins contain an epoxylactol ring attached to the pregnane skeleton at C(20).



Structures of the Side Chains

The δ-lactone structure has been shown by transformations [7, 8] amounting either to catalytic hydrogenation with the hydrogenolysis of the allylic 27-hydroxy group, leading to deoxy derivatives, or to the ozonolysis of the α,β-unsaturated lactone grouping, and the formation in both cases (from the hydroxy and the deoxy derivatives) of one and the same β-hydroxy ketone (XVIII) enabled the primary OH group in withaferin to be assigned to position 27. The upfield shift of the signal from the 21-CH_3 group in the PMR spectrum [doublet in (XIX) at 1.08 ppm to 0.97 ppm in (XX)] confirmed the position of the secondary CH_3 group at C-20 in withaferin A. Hydrogenation of the Δ^{24} bond of the 27-deoxy analog is far slower than that of the Δ^{2} bond, and subsequent alkaline treatment and lactonization leads to epimerization at C-25, as has been shown on deuterated specimens [7]. In chemical transformations of the 2,3-dihydrowithanolides the formation of the 27-methyl ether (XXI), which is unusual for alkaline conditions, is observed, and this has been explained [7] as a process involving the elimination of an OH group accompanying the Michael addition of a molecule of solvent by the following mechanism:



The 27-deoxy analogs of withaferin A have two methyl groups on a tetrasubstituted double bond, giving in the PMR spectrum a broadened six-proton signal at 1.90 ppm, which is characteristic for all the withanolides with a similar substitution of the δ-lactone in the side chain. The presence of a $-CH_2OH$ grouping at C-25 in withaferin A (4) and also in the jaborosalactones (37-41) [16, 31, 30] is shown in the PMR spectrum in the form of a two-proton singlet from the two equivalent methylene protons at 4.35, or 4.90 for CH_2OAc.

The signal from the C-22 proton in the PMR spectrum is extremely characteristic for all the withanolides [9, 16, 29-34], and its shape and position are appreciably affected by substituents at C-20, C-17, and C-23. In actual fact, in compounds having no OH groups at C-20 the same multiplicity of the signals at 4.22-4.40 ppm is observed in the form of a doublet of triplets with constants of 12 and 3.5 Hz caused by coupling with the three vicinal protons at C-20 and C-23 (1-5, 23, 27, 28, 30, 37-41, 43; see Table 2), which confirms the constant stereochemistry of the withanolides at C-22. The doublet of triplets from the C-22 proton appears in a weaker field when such a descreening group as OH at C-17 is near by. In the case of the 17α-hydroxy withanolides (6, 20, 24, 29, 31, 32) [9, 10], this signal appears in the same form at δ 4.63-4.7 ppm. The presence of a third hydroxy group at C-17 has been shown by the reaction of the 2,3-dihydro derivatives with trichloroacetyl isocyanate, in which derivatives are formed for which a low-field signal from the NH of the carbamate residue (δ 8.43 ppm) is characteristic. The localization of an OH group at C-17 is shown by dehydration with the aid of $SOCl_2$, the $\Delta^{17(20)}$ and Δ^{16} derivatives formed showing the same position and shape of the signals from the C-22 proton. To determine the orientation of the 17-OH group a comparison of the values of Δδ in the NMR spectrum in $CDCl_3$ and C_5H_5N has been used. As is well known, in saturated cyclic systems protons present in the 1,3-diaxial positions to an OH group show an upfield shift by 0.2-0.4 ppm ($\Delta\delta^{CDCl_3}_{C_5H_5N}$). In the present case, for the 18-H, 21-H, and 22-H signals the values of Δ are −0.01, −0.16, and −0.2 ppm. Although in actual fact

TABLE 2. Characteristics of the PMR Spectra of the Withanolides and Nicandrins

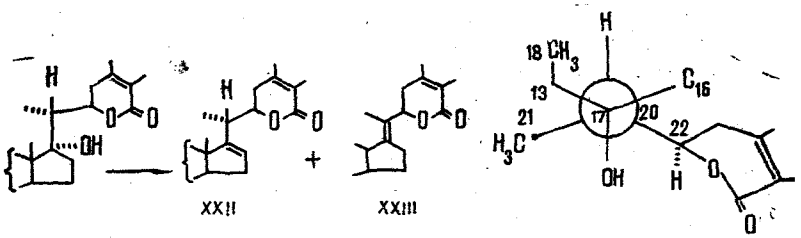
Number of the compound in Table 1	2–H	3–H	4–H	6–H	7–H	22 H	26–H	21–CH_3	18–CH_3	19–CH_3	27 and 28–CH_3	Other signals
1	6,20d 10	7,08 dd 10;6	3,76dd 6	3,23 4		4,37 dt 12;3,5		0,98 d 6	0,68s	1,39 s	1,91	
2	6,21 d 10	7,07 d 6	3,76d 6	3,21 4		4,31 dt 11;3		0,97 d 6	0,69s	1,41s	1,11 d 1,21d 6 6	
3	6,23d 10	7,00 dd 10;6	3,80 d 6	3,26 4		4,41 dt 12;5		1,10 d 7	0,79s	1,43s	1,91	15—H 5,58
4	6,18 d 10	6,97 dd 10;6	3,75 d 6	3,20 4		4,40dt 12;3,5		0,97d 6,5	0,67d	1,39s	2,03	25–OCH_2 4,35s
5	6,18 d 10	6,95 dd 10;6	3,75 d 6	3,23		4,50		1,02d 7	0,8s	1,41s	1,91	
6	6,26 d 10	7,08 dd 10;6	4,69 d 6	3,25 4		4,7 dt		1,01d 7	0,71s	1,40s	1,90	
7	6,21 d 10	6,97 dd 10;6	3,77 d 6	3,25 4		4,25dd 12;5		1,27s	0,83s	1,41s	1,91	
8	6,20 d 10	6,96 dd 10;6	3,75 d 6	3,25 4		4 16 dd 10,5;4		1,26s	0,86s	1,40s	1,17 d 1,21d 6 6	
9	6,29 d 10	7,09 dd 10;6	4,69 d 6	3,26 4		4,29 dd 12;6		1,26s	0,86s	1,39s	2,06	25–OCH_2 4,90
10	6,26 d 10	7,06 dd 10;6	4,68 d 6	3,31 4		4,21 dd 12;6		1,26s	1,00	1,41s	1,95	
11	6,27 d 10	7 08 dd 10;6	4,68 d 6	3,26 4		4,51 dd 14,5;3		1,25s	0,83	1,38	1,95	
12	6,41 d 10	7,3 dd 10;6	4,01 d 6	3,53d 2	3,83d 9	4,36 dd 12;5		1,40s	0,95s	1,88s	1,68 1,88	
50	6,30 d 10	7,08 dd 10;6	4,66 d 6	3,31 4		4,78 dd		1,41 s	1,08s	1,41s	1,89 1,95	
15		5,62 dt 10	6,08 dt 10	5,71		4,25 dd 11,5;4,5		1,30 s	1,07s	1,40s	1,87 1,94	
17		5,64dt 10	6,08dt 10	5.70t		4,66dd 10;5		1,32 s	1,07s	1,39s	1,88 1,95	
14	5,83dq 10;3,1	6,76 dq 10;5;2,5		5,60 8		4,21dd 10;6,5		1,30s	1,05s	1,25s	1,91	
16	5,88 dq 10;3;1	6,83 dq 10;5;2,5		5,63 8		4,30 dd 10;6,5		1,31 s	1,07s	1,26s	2,06	25–OCH_2 4,90
18	5,84 dq 10;3;1	6,83 dq 10;5;2,5		5,59 8		4,62 dd 10,5;5,5		1,31 s	1,06s	1,24s	1,90	
19	5,89 dq 10;3;1	6,81dq 10;5;2,5		5,61 8		4,70 dd 11;5		1,32s	1,21s	1,26s	1,93	15—H 5,26 5
21	5,91 dq 10;3;1	6,81 dq 10;5;2,5		5,56 8		4,70t 8		1,26s	1,24s	1,28s	1,90	15—H 3,58 s
31	5,90 dq	6,83 dq		5,62t		4.66dt		1,15d	1,03	1,25	2,07	15—H 5,25 25—OCH_2 4,93
29	5,90 dq	6,94 dq		5,68t		4,70dt		1,06d 7	0,85s	1,25	2,07	25–OCH_2 4,90
34	5,92 dq 10;3;1	6,86 dq 10;5;2,5		5,63 8		4,42		1,13	0,87s	1,25		23–H 4,42 25–OCH_2 4,42
30	5,99 dq	6,83 dq		5,86	4,90	4,48 dt 12;3,5		1,05d 7	0,73s	1,26	2,06	25—$OCH_2$4,90
35	5,90dq	6,83 dq		5,62m		4,62 dd 12;4,5		1,51 s		1,30s	1,95	
20	5,91	6,66			5,72	4,63 dt		1,04 d 7	0.85s	1,21s	1,90	
32	6,60 d 10	6,83 dd 10;4,7	4,66 d 4,7	6,00		4,68		1,09d 7	0,98	1,50	1,83 1,97	
33	5,91d 10	6,76 dd 10;4,7	4,64 d 4,7	5,78d 1,5	3,84 d 6	4,25dd 12,5		1,30	0,92s	1,46s	1,85 1,96	
36	6,03dq	6,9 dq	3,15			4,58 dd		1,28		1.25s	1,93	18—H 5,17
26	5.85dq 10;3;1	6,56 dd 10;4,5;3		3,05dd 3,5;2	3,33dd 4,1	4,73dd 3,5;2		0.93d 7	0,80s	1,17	1,97	23—H 5,63 d 2
27	5,81	6,61		3,06 d 4	3,32dd 4;1	4,46dt 12;3,5		1,04 d 7	0,78s	1,18s	2,06	25—OCH_2 4.9s
42	5,88 dt 10	6,62 m		3,22 d	4,0m	3,87 m	4,98s	1.24d			1,33 1,35	15—H 4,0 m 16—H 7,38d
43	5,81 dq 10;3;1	6,60 dq 10;4,5;3		3,08 d 4	3,40dq 4;1	4,40 dt 12;3,5		0,98d 6	1,11s	1,24s	1,92	

TABLE 2. (cont.)

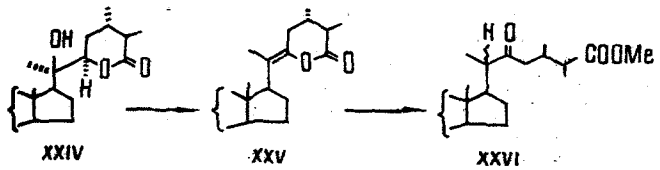
Number of the compound in Table 1	2-H	3-H	4-H	6-H	7-H	22-H	26-H	21-CH_3	18-CH_3	19-CH_3	27 and 28-CH_3	Other signals
48	5,85d 10	6,60dq 10;4,5;3		3,08d 4	3,42d 4;1	4,55m		0,9d 7	1,13s	1,26s	1,52 1,58	
44	5,67d 10	6,61dd 10;4,8;3		2,92dd	3,14	3,92m	4,95d 7	0,85d 6	0,7s	1,32s	1,27 1,07	
45	5,56d 10	6,52dd 10;5;3			3,05dm 4		4,92d 7	0,72s	1,04s	1,32s	1,16 1,27	
46	5,96d 10	6,50dd 10;3,4;8		3,03d 5	3,17dd 5	4,15m	5,36s	1,0d 6	0,60	1,45	1,18 1,40	
47	5,8d 10	6,6dq 3;4	2,76	3,3	3,0	3,8d 7	5,0	1,0d 7			1,4	
24	5,81dq 10;3;1	6,60d 10;4,5;3		3,06d 4	3,34dd 4;1	4,63dt 8;5;3		1,04d 7	0,85s	1,18s	1,90	
28		5,52		2,97d 4	3,31dd 4;1	4,41dt 12;3,5		1,01d 7	0,75	0,85	1,92	1-H 3,68
39	6,06d 11	6,8m		4,38				0,99d 7	0,71	1,12	2,04	25-OCH_2 4,38
40	5,96d 11;2	6,8m		5,22 5		4,46dt 12;4		1,05d 7	0,78	1,37	2,12	25-OCH_2 4,93
41	5,90d 10	6,8m						0,84d 6	0,68	1,66	2,12	25-OCH_2 4,73

*The upper lines give the chemical shifts (ppm) and the lower lines the coupling constants (Hz); s) singlet; d) doublet; t) triplet; dd) doublet of doublets; dt) doublet of triplets; q) quartet; m) multiplet; dq) doublet of quartets.

the 17α-OH and 22α-H are not included in such a system, it is considered probable that such a rigid conformation is preferred for the C(17)-C(20) bond in which the 17-OH group and the 22-H atom are located with respect to one another in a position similar to the 1,3-diaxial position, which causes a solvent shift for the 22-H signal [9].



In the withanolides having a 20-OH group (7-10, 12, 14-16, 25, 33) [10, 16, 18, 19, 33-37], the 22-H signal appears in the PMR spectrum in the form of a doublet of doublets at 4.25-4.30 ppm with constants of 12 and 5-6 Hz. For the 20-trichloroacetylcarbamates characteristic singlets are found in the PMR spectrum at δ 8.70 ppm, and the signals from the 21-CH_3 and 22-H protons are shifted downfield (δ 1.30 and 4.86 ppm, respectively) [18]. To show the α-orientation of the 20-OH group a comparison of the positions of the signals in the PMR spectra from the C-18 CH_3 and C-21 CH_3 groups with the corresponding signals of 20α- and 20β-hydroxycholesterols has been used as a criterion. Chemically, the presence of the 20-hydroxy function is shown by the dehydration of 24-25-dihydroderivatives of the withanolides.



For withanolides (11, 13, 17-19, 21) containing simultaneously 17α and 20α-hydroxy groups [10, 11, 18], the signal from the C-22 proton has the form of a doublet of doublets and is shifted downfield to δ 4.70 ppm through the descreening influence of the 17α-OH group, and the signal from the 21-CH_3 group is shifted to δ 1.25 ppm - through the descreening influence of the 20-hydroxy group. The presence of two tertiary OH groups has been shown by esterification with trichloroacetyl isocyanate in the manner described above.

Particular interest is presented by the presence in nature of 14-hydroxywithanolides, since such substitution is characteristic for cardenolides, bufadienolides, and ecdysones. A proof of the presence of a 14-OH

group in (5) [32] consisted in obtaining $\Delta^{8(14)}$ and Δ^{14} dehydration products. It is also found that the signal from the C-18 CH_3 group is shifted downfield by 6-7 Hz as compared with the 14-deoxy analogs. The presence of two OH groups at C-20 and C-14 (10) [10] is shown by reaction with trichloroacetyl isocyanate when, as in the case of the mono-20-hydroxy derivatives, the signal from the 21-CH_3 group is descreened and is found at δ 1.25 ppm. Thanks to the appreciable influence of the 14α-hydroxy group on the C-18 CH_3, the signal of the latter is shifted downfield as compared with withanolide D (7) by 0.17 ppm and appears at δ 1.00 ppm.

14α,17β,20-Trihydroxywithanolides (13, 22, 50) with the α stereochemistry of the side chain, which is unusual for natural steroids, have also been isolated [10, 11, 18, 32, 40].

In withanolides with a 23-hydroxy group in the δ-lactone ring (26, 34) [17], the substitution at C-23 substantially affects the nature of the 22-H signals in the PMR spectrum. Although the shape and position of the signal from 22-H in (34) are similar to those for other withanolides (doublet of doublets at δ 4.80 ppm), nevertheless, in contrast to the 20-hydroxywithanolides, in which the signal is split because of coupling with the two protons at C-23 into signals with constants of 12 and 5 Hz, in this case the coupling constants are considerably smaller (3.5 and 2 Hz) solely because of axial–axial interaction.

The influence of the acetate group at C-23 on the positions of the signals of the protons close to the substituent in position 23 has been studied. It was found that the signals from the 18-CH_3 and 21-CH_3 groups are shifted upfield, while the signal from the 22-H is shifted downfield by 0.4 ppm. As a model shows, the 23-acetate group restricts rotation around the C(20)–C(22) bond and, as a result, in the most preferred conformation the 18- and 21- protons prove to be more remote from the C-23 substituent (34) than in its hydroxy analog, while the 22-H is present closer to the 17α-OH group and, thus the latter descreens it. This interpretation is confirmed by a comparison of PMR spectra in $CDCl_3$ and in C_5H_5N.

As is well known, the influence of pyridine is due to a hydrogen bond with a hydroxylic oxygen. In the 23-hydroxy compound (24), pyridine coordinates with the 23-OH group and interferes with rotation about the C(20)–C(22) bond in such a way that the distance between the 23-OH and 18-CH_3 groups increases and the signal from the 18-CH_3 group shifts upfield; correspondingly, the protons close to the 23-OH and 17-OH groups are considerably descreened and their signals shift downfield (21-CH_3, 22-H). In the 23-acetate, where pyridine does not coordinate with the acetoxy group, the signals from the 21-CH_3 and 22-H protons (close to the 17-OH group) are shifted downfield, while the signal from the 18-CH_3 group does not change its position.

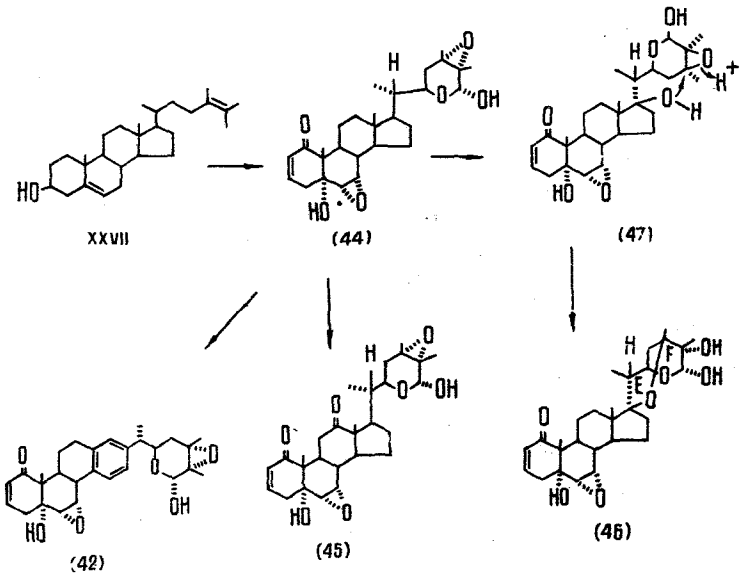
Withaphysalins A and B (35, 36) differ somewhat in the nature of their side chains [39]. Although they also contain an α,β-unsaturated δ-lactone ring at C-20, in each of them the 20-hydroxy function is included in an 18, 20-lactone grouping. In these circumstances, the 22-H atom gives a doublet of doublets in the PMR spectrum at δ 4.65 ppm. The presence of an oxygen atom at C-20 affects the position of the signals from 21-CH_3 group in the weak field at 1.51 ppm in the form of a singlet. The conclusion of the presence of an 18,20-lactone ring is made on the basis of the absence of a signal from the 18-CH_3 group and the strong descreening of the 21-CH_3 group. The very existence of an 18,20-lactone ring determines the stereochemistry at C-17; the possibility of a 17α side chain is excluded. So far as concerns the configuration at C-20, although both possible orientations of the OH group (α and β) permit ring closure with the formation of a γ-lactone, the α stereochemistry is confirmed by biogenetic arguments: in all 20-OH withanolides, just as in the physalins, the configuration at C-20 is α. The stereochemistry at C-22 is the same as in all the withanolides (see below). The presence of a 14α-OH group in (35) is confirmed by its dehydration to a $\Delta^{14(15)}$ derivative and the stereodirected peracid epoxidation of the Δ^5 bond on the corresponding derivative to an α-epoxide, which is connected with the α configuration of the 14-OH group. It is considered that the 14α orientation is preferable biogenetically by analogy with the 14α-hydroxywithanolides (5, 10, 13, 22). Withaphysalin B (36) has in the same 18, 20 position a lactol grouping which, on oxidation with CrO_3, is converted into a γ-lactone. The structure of the acetate of the lactol is confirmed by the low-field position of the signal from the CHOAc group at δ 6.17 ppm.

In the nicandrins (44-47) [20], the lactone ring in the side chain that is characteristic of the withanolides is replaced by an epoxylactol group, and its oxidation leads to a 24,25-epoxywithanolide. A study of the structure of the nicandrins by X-ray structural analysis [20] has shown that in ring E the C(23)-C(26) atoms are located in approximately the same plane as the 24,25-oxide ring, O(22) lies in the same plane, and C(22) is below the main plane of the other five atoms. Nicandrin (45) differs from (44) by the presence of a carbonyl group at C-12 (which is analogous to withanicandrin) [20, 21, 40], and this is shown in the appearance of a signal from the 18-CH_3 group in the PMR spectrum in a considerably weaker field than for (44), at δ 1.04 ppm. Nicandrin (47) has an α side chain that is unusual for natural steroids and a 17β-hydroxy group. The assignment of the tertiary OH group to C-17 is based on the use of a shift reagent and on double-resonance technique [22]. The 17-OH group determines the position of the signal from the 18-CH_3 group in the PMR spectrum of (47) in a weaker field (δ 1.10 ppm) as compared with 17-deoxynicandrin (44) (δ 0.70 ppm).

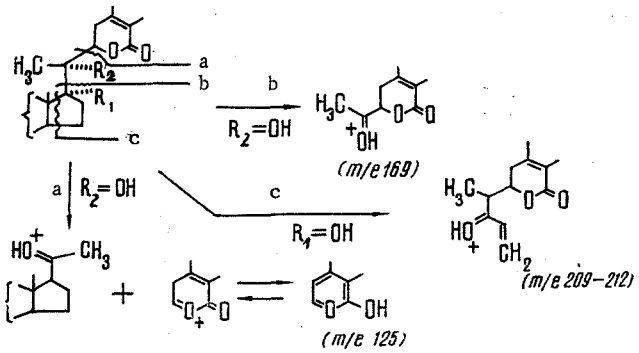
Compound (47) is apparently a biosynthetic precursor of (46), which contains a dioxabicyclo[3,3,1]nonane system at C-17 with an iso side chain. X-ray structural analysis of (46) showed [20] that the 17−20 bond is axial (α), and the C(17)−O bond is equatorial, in contrast to (44), which has a 17β equatorial side chain. In the dioxabicyclo[3,3,1]nonane system, ring E has a slightly distorted boat conformation with the C(17) and C(23) atoms below the main plane of the ring. The substituents C(16) and C(28) are equatorial in relation to the ring and C(13), C(21), O(22), and C(25) are axial.

Ring F assumes the chair conformation with the equatorial substituents O(26), C(27), and C(28) and the axial substituents O(17), C(20), and O(25). The biosynthetic relationships between the nicandrins (44-47) are shown in the Scheme. Their possible precursor is 24-methyl-24,25-dihydrocholesterol, the successive oxidation of which leads to (44) → (45) (47). Here, (46) may be formed by intramolecular opening of the 24,25-epoxide ring by the 17β-OH group. The stereochemistry of (46) agrees with the trans-diaxial opening of the oxide. The hypothesis has been put forward that (46) is also a biosynthetic precursor of an extremely unusual nicandrin that has been detected in nature with an aromatic ring D (42). Its biosynthesis includes the oxidation of the angular 18-CH_3 group, the cleavage of the C(13)−C(17) bond, and C(18)→C(17) recyclization [21-25]. The structure of (42) was assigned from the results of an x-ray structural analysis [24] and has been confirmed by chemical transformations into Δ^{24}-lactones forming analogs of the withanolides. The PMR spectrum of (42) differs from those of the other nicandrins only by the presence of signals in the weak-field region at δ 7.38 and 7.0 ppm.

In addition to PMR spectra, an extremely informative method for determining the structures of the side chains of the withanolides is mass spectrometry since the direction of fragmentation under electron impact is determined by the number and positions of the OH groups in the side chain and by the steroid skeleton. Three directions of fragmentation are possible: a, b, and c. In the absence of 17- and 20-hydroxy groups and, in particular, in the withaphysalins (35, 36) route "a," i.e., cleavage of the C(20)−C(22) bond predominates in the mass spectra of the Δ^{24} compounds, and the strongest peaks are those with m/e 125, $M^+ - 125$, and $M^+ - 125 - 18$. The presence of an ion with m/e 127 shows the presence of a saturated lactone grouping. Weaker peaks are formed on cleavage by route "b," i.e., at the C(17)−C(20) bond: m/e $M^+ - 153$. This type of fragmentation is characteristic of compounds (1-4, 23, 27, 28, 30, 37-41, 43).



The presence of a 20-OH group facilitates the cleavage of the C(20)−C(22) bond, and therefore the 20-hydroxy-Δ^{24}-withanolides (7-9, 12, 14-16, 25) are characterized by the strongest fragments with m/e 125 and $M^+ - 125$ and fragments with a very low intensity having m/e 169 and $M^+ - 169$ (route "b") [37]. The absence of a 20-hydroxy group and the presence of a 17-hydroxy function intensifies fragmentation by route "c": cleavage at the C(13)−C(17) and the C(14)−C(15) bonds. The absence of peaks with m/e 209 (212) leads to the conclusion that a 17-hydroxy group is absent. In the mass spectra of compounds (6, 20, 24, 29, 31, 32) they are extremely intensive. The presence of OH groups at C(17) and C(20) facilitates fragmentation at the C(17)−C(20) bond, i.e., route "b." Consequently the presence in the mass spectra of compounds (11, 17-19, 21) of an intense ion with m/e 169 confirms the position of the OH groups in the molecule at C(20) and C(17).



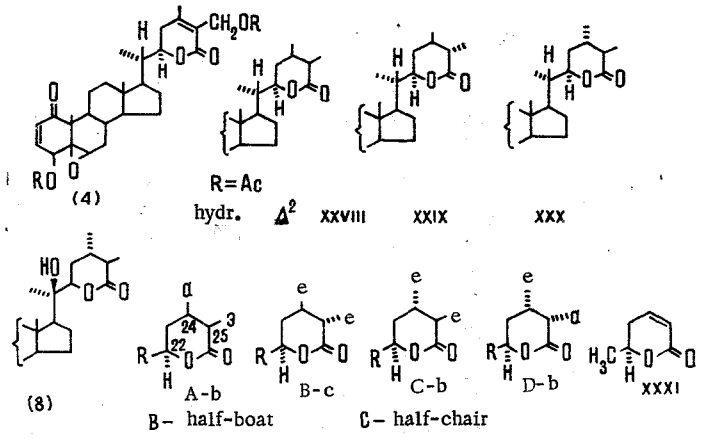
In the 17,23-dihydroxywithanolide (34), the main peak in the mass spectrum is due to cleavage by route "b" and the elimination of water: m/e 295 ($M^+ - 157 - 18$). There are also two considerable fragments with m/e 342 and 324 which are assigned to the cleavage of the lactone itself at the C(22) – C(23) bond and the O – C = O grouping [17]. In compounds containing 14-hydroxy or 14,20-dihydroxy groupings (5, 10, 13, 22), in contrast to the 17,20-diols, where cleavage at the C(17) – C(20) bond is predominant, the peak arising through the cleavage of the C(20) – C(22) bond with m/e ($M^+ - 125 - 18$) are intensified [10].

Conformational Features of the Side Chains

A conformational study of six-membered lactones by x-ray structural analysis has shown that the carbonyl group, the ester oxygen atom, and the two neighboring carbons atom lie in a common plane, i.e., the saturated lactone ring must have either the half-chair or the half-boat conformation. Both conformations, with small differences in energy, have been found in the crystalline state [33]. A convenient method for determining the preferred conformations of saturated lactones in the withanolides (2, 8, XXVIII-XXX) has proved to be circular dichroism, since it was important only to know the configuration at C-22. In the jaborosalactones (37-41), having the same side chains as the withanolides (3, 4), the R configuration at C-22 was established by comparing the CD curves with those of the lactone of parasorbic acid (XXXI), showing a positive Cotton effect at 250 nm [42]. All the withanolides having similar CD spectra are therefore considered as possessing 22(R) stereochemistry. Exceptions are compounds 48 and 52 [38, 47]. In view of the 22R configuration and the equatorial orientation of the bond between the lactone and the steroid skeleton, from a consideration of models it may be concluded [33] that a positive sign of the Cotton effect in the region of the $n \rightarrow \pi^*$ transition of saturated lactones at 215 nm must correspond to a half-chair conformation, and a negative sign to a half-boat conformation. In the lactone ring of (XXVIII), a compound obtained by the catalytic hydrogenation of (4), the CH_3 groups at C-24 and C-25 must be present in the cis position. The sign of the Cotton effect for (XXVIII) in the 215 nm region is negative and, therefore, the existence of two half-boat conformations is possible with the methyl groups β-oriented in A – b and α-oriented in D – b.

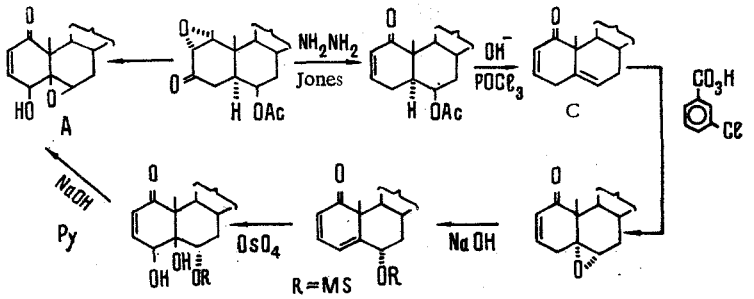
The choice between these conformations was made after the use of the well-known idea of the difference in chemical shifts due to solvents in the PMR spectrum, $\Delta\delta^{CDCl_3}_{C_6H_5}$, from which it is clear that the proton of the methyl group present in the α-position to the keto group has a considerable value of $\Delta\delta$ (in the strong-field direction) if this group is axial and a very small one (in the direction of weak fields) if it is equatorial. The small solvent shifts $\Delta\delta^{CDCl_3}_{C_6H_6}$ of similar magnitude for the 25-CH_3 group in (XXVIII, XXIX, XX) of 4.5, 5, and 4 Hz permit the conclusion that the 25-CH_3 groups in the neighborhood of the lactone carbonyl are similarly oriented in all the compounds mentioned, and this orientation is equatorial.

Taking these facts into account, for the lactone (XXVIII) the half-chair conformation A – b was finally selected. The positive sign of the Cotton effect in the region of the $n \rightarrow \pi^*$ transition for (XXIX) leads to the conclusion that (XXIX) has the B – C half-chair conformation in which the CH_3 groups at C-24 and C-25 are trans-diequatorial. It is possible that (XXIX) is the equilibrium product of the hydrogenation of (4). On the basis of the negative sign of the Cotton effect in the region of the $n \rightarrow \pi^*$ transition and the solvent shift in the PMR spectrum $\Delta\delta^{CDCl_3}_{C_6H_6}$, approximately 4 Hz, the lactone ring in (8) has the C – b conformation. In the all the the proposed structures for the lactones (XXVIII-XXX, and 8) (A – b, B – c, C – b), 1,3-diaxial interactions are reduced to a minimum.

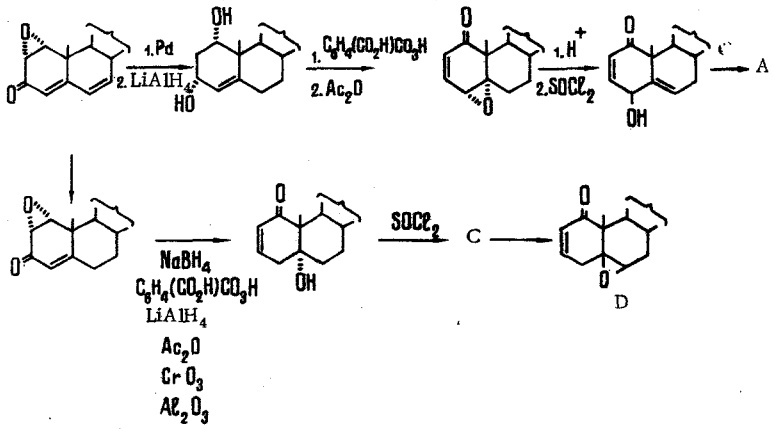


Syntheses of Withanolides

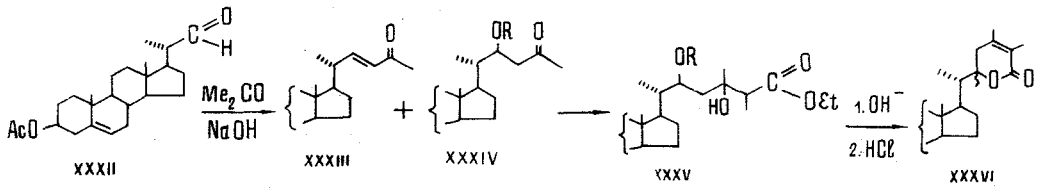
The first synthetic investigations in the field of the withanolides were directed to the creation of the dihydroquinoid fragment in ring A and the epoxide formation in ring B. Japanese workers [43, 44] have developed a stereoselective synthesis of 1-oxo-5β,6β-epoxycholest-2-en-3β-ol as a model of compound (A).



Lavie et al. [45] have put forward a partial synthesis of the structures of rings A/B containing, in addition to substituents of types A and C, also B and D, not only in the cholestane but also in the 17β-acetoxyandrostane and 20β-acetoxypregnane series.



A partial synthesis of the side chain of the withanolides has been performed [46] from the 20-aldehyde (XXXII) by condensation withe acetone and the use of the Reformatsky reaction with ethyl α-bromopropionate, cyclization of the resulting dihydroxy ester giving the α,β-unsaturated δ-lactone ring at C-20. The stereochemistry at C(22), evidently so important for the manifestation of biological activity is already present in the stage of study (see Scheme on following page.)



XXXII XXXIII XXXIV XXXV XXXVI

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STRUCTURE OF THE PECTIC ACID OF Matricaria chamomilla

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UDC 547.917

A water-soluble polysaccharide complex has previously been isolated from the racemes of Matricaria chamomilla L. (German chamomile) and its mono- and polysaccharide composition has been studied [1-4]. We have now investigated the structure of the pectic acid found in the fractionation of the initial complex [3].

In the first stage of the investigations, the polysaccharide was subjected to enzymatic hydrolysis. The products were found to contain mono-, di, tri-, and tetragalacturonic acids and galactose, arabinose, and xylose.

Periodate oxidation of the pectic acid at +15°C was complete in 24 h. The consumption of sodium metaperiodate was 0.81 mole per anhydro unit. Consequently, the polysaccharide does not have a strongly branched structure. The oxidation product was isolated from the reaction mixture, $[\alpha]_D$ −85° (c 2% in water), and was hydrolyzed. By paper chromatography (PC), weak spots of galacturonic acid and rhamnose, and also of xylogalactose were found in the hydrolyzate.

The partial acid hydrolysis of the pectic acid was then carried out. A polymer was isolated from the hydrolyzate with $[\alpha]_D$ +347° (c 0.2% in water in the form of the sodium salt). The physicochemical properties and IR spectra of the polysaccharide were close to those of the products of partial hydrolysis obtained previously from the pectin substances [5].

The pectic acid was methylated by Hakomori's [6] and Purdie's [7] methods after preliminary esterification with a 1 M solution of sulfuric acid in methanol and reduction of the carboxylic ester groups with sodium tetrahydroborate to primary alcohol groups [8]. Chromatography of the methylated polysaccharide on Al_2O_3 gave only one spot, showing homogeneity. The IR spectra contained no absorption bands in the region of hydroxy groups. This demonstrates that the process of methylation had gone to completetion.

In an investigation of the degradation products from the methylated polysaccharide by PC, a complex set of methylated monosaccharides was obtained, and therefore no further study was continued after they had been separated on a coumn of cellulose [7].

The isolation of considerable amounts of fully methylated L-arabinose and D-xylose indicates that the corresponding sugar residues form a covalent bond with the main skeletal structure of the polysaccharide in the form of individual branches. The same can be said about the D-galactose isolated from the degradation products in considerable amounts.

The isolation of 2,3,6-tri-O-methyl-D-galactose as the main component indicates the presence of a skeletal structure consisting of D-galacturonic acid residues connected by 1→4 bonds. The considerable positive specific optical rotation of the polysaccharide shows the α configuration of the glycosidic bond. In the molecule, the galacturonic acid residues are present in the pyranose form, as is shown by the IR spectrum, which has absorption bands at 1000-1110 cm^{-1} (vibrations of a pyranose ring) [9, 10]. The isolation of 3,4-di-O-methyl-L-rhamnose permitted the assumption that rhamnose is included in the main polysaccharide chain by 1→2 bonds.

Thus, the main polysaccharide chain of the pectic acid from German chamomile consisted of residues of α-D-galacturonic acid in the pyranose form linked by 1→4 glycosidic bonds. Single branchings consisting of the neutral monosaccharides galactose, arabinose, and xylose are possible.

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