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WITHASTEROIDS OF *Physalis*.

IV. 28-HYDROWITHAPHYSANOLIDE. ^{13}C NMR SPECTRUM OF 14-α-HYDROXYWITHASTEROIDS

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A new withasteroid has been isolated from *Physalis viscosa* L. — 28-hydroxywithaphysanolide, with mp 234°C, composition $C_{28}H_{38}O_8$. The ^{13}C NMR spectra of a number of withasteroids have been investigated. For 28-hydroxywithaphysanolide we propose the structure 4β,14α,17β,20R,28-pentahydroxy-1-oxo-22R-witha-2,5,24-trienolide. The corresponding corrections have been made in the structural formulas of withaphysanolide and physalactone.

Structure of 28-Hydroxywithaphysanolide (I)

From an aqueous extract of the epigeal part of *Physalis viscosa* L. [1] we have isolated a new polar withasteroid (I) with the composition $C_{28}H_{38}O_8$ (M^+ 502). A strong maximum in the UV spectrum at 212 nm (log ε 4.10) and the presence in its mass spectrum of ions with m/z 185 and 141 indicate the presence of an additional oxygen function in an unsaturated lactone ring. The peak of the ion with m/z 185 has the maximum intensity, which shows the cleavage of the bond between C-17 and C-20 that is characteristic for withasteroids containing a diol grouping at the position of cleavage [2]. With acetic anhydride, the new withasteroid (I) formed a di-O-acetate (II). When compound (II) was oxidized with the Jones reagent, a lactone (XIII) and 4β-acetoxy-14α-hydroxy-1,17-dioxoandrostane-2,5-diene (XIV) were obtained. Precisely the same derivative of androstane-2,5-diene was obtained previously by the oxidation of withaphysanolide acetate (III), but compound (XIV) was ascribed a somewhat different structure [2]. The ease of degradation of (II) by chromium trioxide is a new confirmation of the presence of a diol grouping at C-17 and C-20.

In the PMR spectrum, the positions of five one-proton signals depended to a considerable degree on the temperature of the solution of the substance which showed that they belonged to five hydroxy groups. Three of them appeared at 30°C in the form of singlets (tertiary OH groups) at 5.16, 6.19, and 6.97 ppm, and the other two were split into a doublet (7.23 ppm, ^3J = 4.4 Hz), and a triplet (7.05 ppm, ^3J = 12.1 Hz). In the spectrum taken at 50°C, the same signals appeared at 4.60, 5.81, 6.84, 6.86, and 6.76 ppm, respectively. The doublet and triplet splitting of the weak-field signals is due to spin–spin coupling between the hydroxylic protons and the methine and methylene protons arranged geminally to them. Consequently, in (I) the two oxygen functions are represented by secondary and primary hydroxy groups. The components of the two doublet signals of the methylene proton of the CH_2OH group were additionally split through coupling with the proton of the hydroxy group (triplet at 7.05 ppm). They appeared in the form of quartets at 4.40 ppm ($^2J_{gem}$ = 14.5 and $^3J_{CHH'-OH}$ = 6.5 Hz) and 4.17 ppm ($^2J_{gem}$ = 14.5 and $^3J_{CHH'-OH}$ = 5.6 Hz). In the spectrum of the acetate (II) the signals were shifted downfield (4.75 and 5.01 ppm) and appeared in the form of two AB doublets with $^2J_{gem}$ = 13.6 Hz.

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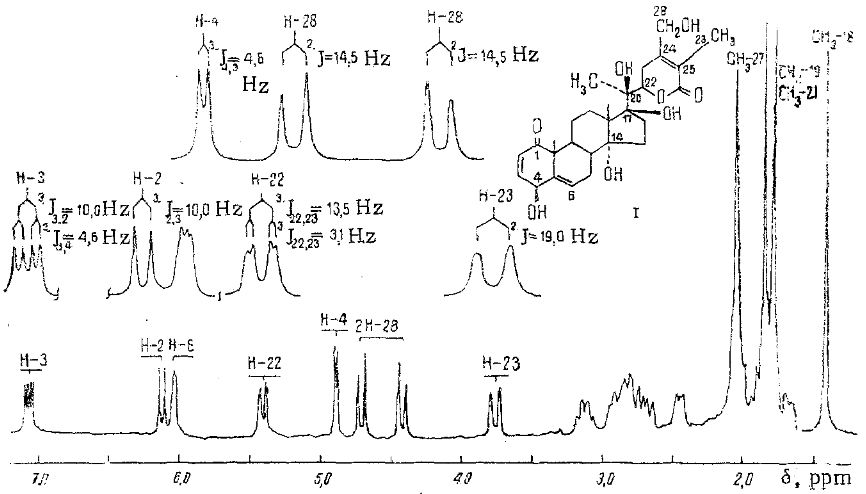


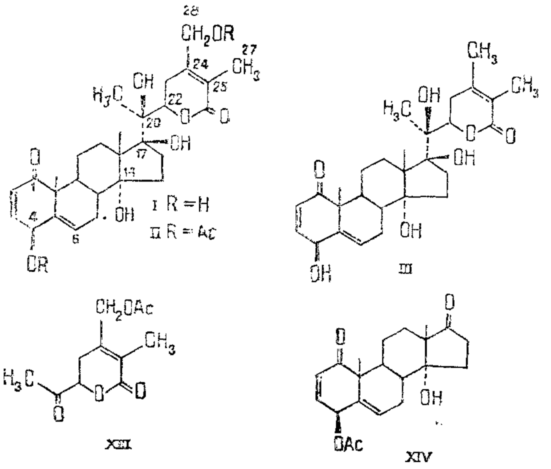
Fig. 1. PMR spectrum of 28-hydroxywithaphysanolide (I) in deuteropyridine with the addition of trace amounts of trifluoroacetic acid.

In addition to this, in the region of resonance of the protons of the methyl groups there were four signals each of which corresponded to three proton units: singlets at 1.41, 1.79, and 1.86 ppm (CH_3-18, -19, and -21) and a doublet at 2.05 ppm with J = 2 Hz. Of them, only the last signal corresponded in the value of its chemical shift and the nature of its splitting to a methyl group at a double bond. Consequently, in the lactone ring of compound (I), unlike withaphysanolide (III) there is only one methyl group. These results indicate that the additional oxygen function in the lactone ring that was mentioned above consists of a hydroxymethyl group: 27-CH_2OH or 28-CH_2OH.

A quartet signal at 5.44 ppm with ^{3}J = 13.5 and 3.1 Hz belongs to the H-22 proton of the lactone ring, which interacts vicinally only with the two protons at C-23, and this agrees well with the presence of a diol grouping at C-17 and C-20 of the compound (I) under investigation. A doublet at 6.13 ppm with ^{3}J = 10.0 Hz (H-2) and a quartet at 7.06 ppm with ^{3}J = 10.0 and 4.6 Hz (H-3) are characteristic for isolated protons present in ring A on a double bond conjugated with a carbonyl group [3, 4]. Consequently H-3 interacted vicinally not only with H-2 (^{3}J = 10.0 Hz) but also with one proton at C-4 (^{3}J = 4.6 Hz), and there must be a secondary hydroxy group attached to this carbon atom. The H-4 signal appeared in the form of a triplet at 4.88 ppm with $^{3}J_{3,4} + {}^{3}J_{C_4H\underline{OH}}$ = 9.2 Hz, and on passing to the acetate (II) it underwent a considerable paramagnetic shift to 5.91 ppm and appeared in the form of a doublet with ^{3}J = 4.9 Hz. This value of ^{3}J between the H-3 and H-4 protons shows the quasi-equatorial orientation of H-4 and, correspondingly, the β-axial orientation of the OH group at C-4.

The PMR spectrum of hydroxywithaphysanolide (I) (Fig. 1) also had a signal corresponding to an olefinic proton. It appeared in the form of a quartet with broadened components at 6.03 ppm, ^{3}J = 2.0 and 5.0 Hz. In the spectrum of the acetate (II), the signal of the olefinic proton was present in a weaker field (at 6.20 ppm), i.e., Δδ for it amounted to 0.17 ppm. This shift is obviously due to the closeness of the acetyl group at C-4 and the olefinic proton. On the basis of this fact, and also in view of the doublet nature of the H-4 signal in the PMR spectrum of the acetate (II) the double bond in the steroid moiety of compound (I) is obviously present at the C-5 and C-6 carbon atoms. In this case, the tertiary hydroxy group may be located at C-14. Thic conclusion agrees well with the above-mentioned fact that the oxidation of the diacetate (II) led to 4β-acetoxy-14α-hydroxyandrosta-2,5-diene-1,17-dione (XIV).

Analysis of the characteristics of the PMR spectra has permitted an approximate determination of the main elements of the structure of the withasteroid (I). Complete information on the structure of this compound and, in particular, information permitting an accurate determination of the position of the primary hydroxy group, was obtained only in an analysis of the characteristics of the ^{13}C NMR spectrum of (I) and a number of related withasteroids. This simultaneously permitted corrections to be made to the structure of some other withasteroids.



Scheme 1

^{13}C NMR Spectra of 14α-Withasteroids

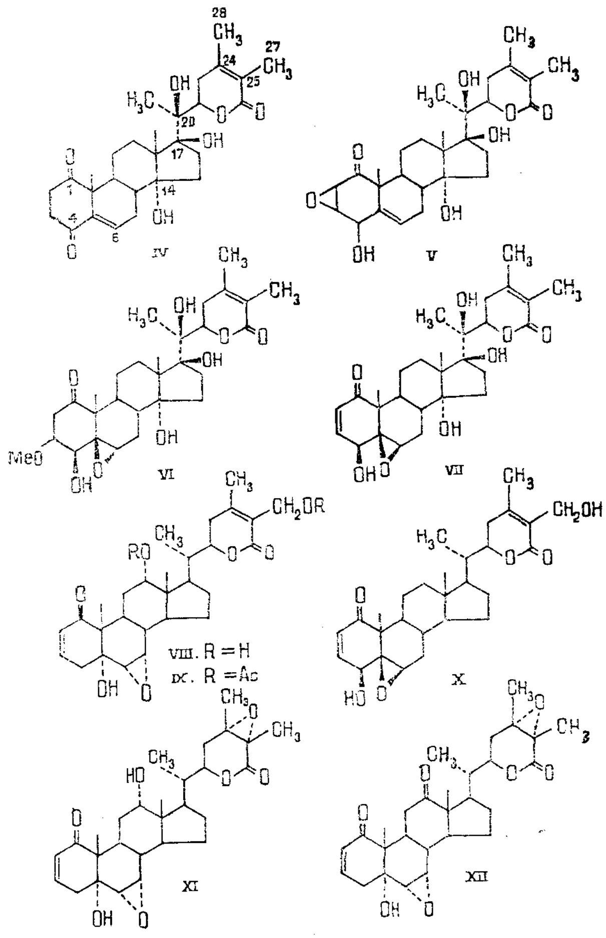
The use of ^{13}C NMR spectroscopy for determining the structures of withasteroids has begun comparatively recently [5-9]. However, even the first investigations showed that the characteristics of the ^{13}C NMR spectra of these oxygen-rich steroid lactones give extremely valuable information. They substantially supplement the conclusion obtained on the basis of the chemical transformations and the combined use of other spectral methods. For example, only on the basis of the use of ^{13}C NMR spectroscopy were the structures of a number of withasteroids from *Withania somnifera* preconsidered; a $\Delta^{8(14)}$ structure has previously been proposed for them. It has now been shown that they do not have a double bond between C-8 and C-14 but contain an α-orientated hydroxy group at C-14 [6, 10]. On this basis the hypothesis has been expressed that withanolide O [11] and physalactone [12] are, probably, also 14α-hydroxy compounds [13].

We have investigated the ^{13}C NMR spectra of the hydroxywithaphysanolide (I) and its diacetyl derivative (II), and also those of withasteroids isolated previously from *Physalis viscosa* L.: withaphysanolide (III) [2], physanolide (IV) [1], the product of the epoxidation of withaphysanolide (V), physalactone (VI) [12], and withastramonolide (VIII) and its diacetyl derivative (IX) [16]. Some of the compounds mentioned are characterized by poor solubility in nonpolar media. In view of this, and for uniformity in the series of compounds studied, the ^{13}C NMR spectra were taken in deuterated pyridine. For comparative investigations we also took 4β-hydroxywithanolide E (VII) [1, 14, 15], withaferin A (X) [17-20], and daturolactones A (XII) and B (XI) [4, 21] isolated from *Datura stramonium*, for which no spectral characteristics obtained in C_5D_5N have been found in the literature. Compounds (VIII-XII) have no hydroxy group at C-14, but, among them, (VIII), (IX), and (XI) contain a hydroxy function at C-12 and (XII) an oxo group on the same carbon atom.

In each concrete case the complete assignment of the signals was carried out by a comparative study of the spectra taken under conditions of complete and partial suppression of spin-spin coupling with protons, and an analysis of the chemical shifts of the carbon atoms using literature information on the ^{13}C NMR spectra of compounds close in structure. The results of the assignment of the signals and the values of the chemical shifts of the carbon atoms of the compounds under investigation are generalized in Table 1.

In the weakest field of the ^{13}C NMR spectrum of hydroxywithaphysanolide (I) at 203.9 and 167.1 ppm there are singlet signals of the carbon atoms C-1 (α,β-unsaturated ketone) and C-26 (lactone in a side chain). Doublets at 130.3 and 145.9 ppm belong to the C-2 and C-3 carbon atoms, respectively. For substance (I) the signal of the C-3 carbon atom is located in a weaker field (by 5 ppm) than the corresponding signals in the spectra of compounds (VIII-XII) (140.5-141.0 ppm). This shift is due to the presence of a hydroxy function at C-4 in the molecule of the withasteroid (I). A doublet at 69.1 ppm is assigned to the last-mentioned carbon atom.

Similar values of the chemical shifts (CSs) characterize the signals of the corresponding carbon atoms of ring A of withaphysanolide (III) and of 4β-hydroxywithanolide E (VII) both of which have a β-hydroxy function at C-4 (see Table 1).



Scheme 2

We have already mentioned the possibility of the location of the double bond in the steroid moiety of the molecule of compound (I) at the C-5 and C-6 carbon atoms. This is also shown unambiguously by the presence in its ^{13}C NMR spectrum of a singlet at 139.3 ppm and a doublet at 128.5 ppm assigned to the C-5 and C-6 carbon atoms, respectively. This conclusion agrees well with the fact that on passing from the withasteroid (I) to its di-O-acetyl derivatives (II) a considerable diamagnetic shift is observed for the C-3 and C-5 signals and a paramagnetic shift for C-2 and C-6. Attention is attracted by the fact that the chemical shifts of the singlet at 139.3 and the doublet at 128.5 ppm in the spectra of the hydroxywithanolide (I) and the withaphysanolide (III) (139.5 and 128.6 ppm) are, respectively, practically identical.

In the ^{13}C NMR spectrum of physanolide (IV) there are singlets at 211.1 and 198.8 ppm which correspond to the carbon atoms of two oxo groups, one unconjugated and one conjugated with a double bond. A singlet at 142.2 ppm and a doublet at 138.8 ppm correspond to the C-5 and C-6 carbon atoms at a double bond. The chemical shifts of these signals differ considerably from those for compounds (I) and (III), which is due to conjugation with the oxo group at C-4 (198.8 ppm). The ^{13}C NMR spectra likewise show that one of the common features in the structure of withasteroid (I-IV) is the presence of Δ^5-double bond.

It is known that in the 14α-hydroxywithanolide series the CSs of the C-14 carbon atoms (in $CDCl_3$) amounts to 82.0-96.0 ppm [6]. In the ^{13}C NMR spectra of the compounds (I-VI) considered here, and also in that of 4β-hydroxywithanolide E (VII), for which the 14α-hydroxy structure has been shown strictly [14, 15, 22], there is a singlet signal the CS of which changes only slightly (81.6-82.1 ppm). This signal we assign unambiguously to the C-14 carbon atom bearing the α-hydroxy group. In view of this, and also in consideration of the presence in the ^{13}C NMR spectrum of compound (III) of signals at 139.5 and 128.6 ppm assigned to the C-5 and C-6 carbon atoms, the corresponding corrections must be made to the structures proposed previously for withaphysanolide [2] and physalactone [12]. As can be seen from the structures of (III) and (VI), these two compounds belong to the α-hydroxywithasteroid series and the double bond not conjugated with the carbonyl group in the withaphysanolide (III)

TABLE 1. Chemical Shifts (ppm) of the Carbon Atoms of Compounds (I-XII) (in C_5D_5N and $CDCl_3$ relative to TMS)

C atom	Compound												
	I	II	II*	III	IV	V	VI	VII	VIII	IX*	X	XI	XII
1	203,9	202,8	202,9	204,0	211,1	209,9	209,5	202,2	203,5	202,2	202,1	203,3	202,8
2	130,3	34,9	134,5	130,4	36,6	58,6	41,0	132,4	129,1	128,8	132,2	129,1	129,0
3	145,9	140,4	140,0	146,1	36,1	56,4	78,7	144,4	140,5	139,6	144,9	140,4	141,0
4	69,1	70,5	70,5	69,2	198,8	73,2	74,8	70,3	37,6	36,7	70,6	37,5	37,7
5	139,3	134,9	133,9	139,5	142,2	141,1	65,0	64,4	73,8	73,1	64,8	73,7	73,9
6	128,5	131,3	131,3	128,6	138,3	127,0	58,9	60,8	55,4	56,9	60,5	56,4	56,3
7	26,5	26,5	26,0	26,6	26,9	26,3	26,5	26,5	56,4	56,2	30,5	56,4	55,6
8	36,6	36,6	36,0	36,8	36,2	36,5	34,6	34,7	36,6	35,7	30,8	36,6	36,1
9	37,3	37,1	36,6	37,5	36,3	36,5	36,6	37,6	29,2	29,5	45,0	29,1	38,4
10	50,1	49,7	49,2	50,3	50,3	49,3	50,9	48,4	51,3	50,4	48,9	51,2	52,2
11	23,2	23,3	22,7	23,3	22,7	22,6	21,6	21,5	30,4	26,6	22,3	30,3	39,0
12	33,1	33,1	32,5	35,2	35,2	35,1	35,0	34,9	71,9	74,9	27,8	72,0	212,7
13	54,9	54,9	54,5	55,0	55,0	55,2	54,9	54,7	43,9	45,9	43,1	43,9	57,9
14	81,9	81,7	82,0	81,9	81,6	82,1	81,7	81,8	47,4	45,1	56,5	47,4	53,5
15	29,9	30,2	29,5	31,2	30,9	30,6	30,2	30,3	23,4	22,9	25,0	23,4	23,7
16	37,2	37,1	37,9	37,3	37,2	37,2	37,1	37,1	26,6	26,6	39,9	26,6	26,9
17	88,4	88,5	88,1	88,5	88,3	88,2	88,1	88,0	43,0	43,6	52,4	43,0	42,8
18	19,8	19,7	19,6	19,7	19,6	19,6	19,5	9,4	11,9	12,3	12,2	11,5	11,3
19	21,1	21,1	21,4	21,2	20,7	21,1	14,9	16,5	14,9	14,5	17,7	14,8	14,7
20	79,4	79,3	79,0	79,4	79,3	79,2	79,2	79,1	39,4	38,4	39,5	39,2	39,7
21	22,5	21,7	21,5	22,6	21,2	21,1	20,7	20,7	12,5	12,3	14,0	12,5	13,3
22	82,8	82,6	81,3	81,7	81,6	82,1	81,6	81,8	78,6	78,1	78,6	76,7	76,5
23	30,9	31,0	30,3	33,2	33,0	32,8	32,9	32,9	30,0	30,0	32,3	28,8	28,8
24	154,4	147,3	146,6	150,6	150,7	150,8	150,8	150,7	153,9	156,7	153,7	59,5	59,7
25	121,0	124,6	124,9	121,6	121,6	121,4	121,4	121,3	127,1	122,0	127,3	63,1	63,2
26	167,1	166,4	165,9	165,7	166,8	166,7	166,7	166,5	166,3	165,1	166,0	169,8	169,8
27	11,9	12,3	12,3	12,6	12,5	12,4	12,4	12,4	56,1	58,0	56,6	13,8	13,9
28	60,9	63,1	62,9	20,2	20,2	20,1	20,1	20,1	19,9	20,6	20,6	17,7	17,7
O—C=O	—	169,9	170,5	—	—	—	—	—	—	69,6	—	—	—
CH$_3$	—	20,4	20,5	—	—	—	—	—	—	20,8	—	—	—
O—C=O	—	170,2	170,2	—	—	—	—	—	—	170,8	—	—	—
CH$_3$	—	21,1	20,6	—	—	—	—	—	—	21,2	—	—	—

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*The spectra of compound (II) and (IX) were taken in $CDCl_3$.

molecule is formed between the C-5 and C-6 carbon atoms. The disputed nature of the structure of physalactone is also discussed in a paper by Begley et al. [3].

On consideration of the results given in Table 1, attention is attracted by the stability of the values of the CSs of the carbon atoms C-(13-18) and C-(20-22), which are close to the 14-, 17-, and 20-OH groups. There is no doubt that this circumstance, indicating an identity of the electronic and spatial environments of these carbon atoms, is due to the uniformity of the configuration of the three OH groups mentioned. At the same time, analysis of the ^{13}C NMR characteristics of 14α,17α,20β- and 14α,17β,20β-trihydroxywithanolides given in the literature [6] shows that the CSs of carbon atoms C-12 (ring D) and C-20 depend on the orientation of the OH group at C-17. If it has the β orientation, then carbon atoms C-12, C-13, C-16, and C-20 undergo a considerable paramagnetic, and the C-14 atom a diamagnetic, influence from this hydroxy group. Basing ourselves on these facts, let us compare the CSs of these carbon atoms in compounds (I), (III), (VI), withanolide E (17β-OH) and withanolide J (17α-OH) (ppm):

Compound	C-12	C-13	C-16	C-20	C-14
28-Hydroxywithanolide (I)	33,1	54,9	37,2	79,4	81,9
Physanolide (III)	35,2	55,0	37,3	79,4	81,9
Physalactone (VI)	35,0	54,9	37,1	79,2	81,7
Withanolide E [6]	34,3	54,5	37,7	80,0	82,9
Withanolide J [6]	26,8	51,0	33,3	77,2	86,1
Δδ	+7,5	+3,5	+4,4	+2,8	−3,6

The characteristics given for compounds (I), (III), and (VI) and withanolide E are similar to one another and differ considerably from those of withanolide J, which has a 17α-OH group. It follows from this that the hydroxywithanolide (I) contains a 14α,17β,20β-trihydroxy function.

As will be shown below, the determination of the position of the primary alcohol group in the lactone ring of withasteroid (I) is connected directly with the study of the CS values of the carbon atoms of the methyl groups.

In the ^{13}C NMR spectrum of compound (I), the quartet signals of four methyl groups appear at 19.8, 21.1, 22.5, and 11.9 ppm. Of these, the first three relate to the CH_3-18, -19, and -21 methyl groups, respectively, which does not contradict information in the literature [6, 5]. This assignment is also in harmony with the facts reported in the literature according to which the CSs of the carbon atoms of methyl groups are practically independent of the nature of the substituents in ring A and the lactone ring [5] and the replacement of a Δ^5-bond by a 5β,6β-epoxy group leads to a diamagnetic shift of the C-19 signal by 5-6 ppm while the CSs of the C-18 and C-21 carbon atoms remain unchanged. It is possible to be convinced of the correctness of what has been said by a comparative analysis of the figures given in Table 1 which clearly show the stability of the CSs of the C-18, -19, and -21 carbon atoms in compounds (I-V) and a substantial change in the CS of C-19 in withasteroids (VI) and (VII), each of which has a 5β,6β-epoxy group.

It is known that in the ^{13}C NMR spectra of withasteroids with an α,β-unsaturated lactone ring the signal of the CH_3-27 methyl group appears in the strongest field and that of the CH_3-28 group in a weaker field [5, 6]. This is precisely the position that the C-27 and C-28 signals (12.6-12.4 ppm and 20.2-20.1 ppm, respectively) have in the spectra of withasteroids (III-VII). The introduction of a primary alcohol group at C-27 and, all the more, its acetylation do not lead to any appreciable change in the CS of CH_3-28 whatever. This is indicated both by the information on 27-hydroxywithanolides in the literature [5-7] and by a comparison with one another of the spectral characteristics of withastramonolide (VIII), its diacetyl derivative (IX), and the two compounds that we are now studying (see Table 1). Consequently, the remaining quartet at 11.9 ppm in the spectrum of compound (I) that has not yet been considered must be assigned to the single methyl group of the lactone ring, namely CH_3-27.

It is known from the work of other authors [5, 6] that in the ^{13}C NMR spectra of 27-hydroxy- and 27-acetoxywithasteroids the CSs of the C-27 atom amount, as a rule (in $CDCl_3$), to 57.0-57.5 and 58.0 ppm, respectively. In the spectrum of the hydroxywithaphysanolide (I) the triplet corresponding to the methylene carbon of the primary alcohol group is located at 60.9 ppm, and in the case of its diacetate (II) it undergoes a paramagnetic shift by 2.2 ppm and is located at 63.1 ppm. It can be seen from Table 1 that for compound (I) the signal of the carbon atom of the 28-CH_2OH group is paramagnetically shifted by ~4.5 ppm relative to the signal of the 27-CH_2OH group in the 27-hydroxywithasteroid series (compare, for example, withastramonolide (VII)). Furthermore, it follows from an analysis of the literature [5-7] and the characteristics of compounds (III) and (VIII) and compounds (III) and (I) (see Table 1) that the replacement of a 25-CH_3 group by a 25-CH_2OH group leads to a considerable paramagnetic shift of the C-25 and C-24 signals. On passing from compound (III) (24-CH_3) to (I) (24-CH_2OH) such a change is observed only for the C-24 carbon.

When the 27-OH group of withasteroids is acetylated, the singlet from C-25 which bears a primary alcohol group undergoes a diamagnetic shift by 3.6 ppm, while the C-24 signal undergoes a paramagnetic shift of approximately the same amount. In the ^{13}C NMR spectrum of the hydroxywithaphysanolide (I) we assign the singlets at 154.4 and 121.0 ppm unambiguously to the C-24 and C-25 carbon atoms, respectively, it being just these carbon atoms that participate in the formation of the α,β-unsaturated system of the lactone ring. In the spectrum of the diacetyl derivative (II), the same carbon atoms are characterized by singlets at 147.3 and 124.6 ppm. It is obvious from this that when the primary OH group of compound (I) is replaced by a O-acetyl group, the singlet from C-24 undergoes a diamagnetic shift by 7.1 ppm and that from C-25 a paramagnetic shift by 3.6 ppm. In other words, the changes in the CSs of the carbon atoms under consideration take place in the opposite sequence as compared with that which was observed on passing from the 27-hydroxywithasteroids to their acetates. In principle, it could be assumed that this nature of change in the CSs of C-24 and C-25 observed on passing from compound (I) to its diacetyl derivative (II) is due to a certain extent to the specific influence of the molecules of the pyridine used as solvent. To exclude this possible factor, we have studied the ^{13}C PMR spectra of the known withasteroid withaferin A (X) in Py-d_5 (the figures for (X) in $CDCl_3$ are given in [5]), and also those of the diacetates (II) and (IX) in $CDCl_3$ (see Table 1) (we isolated the withaferin A from the epigeal organs of the plant *Withania somnifera* Dun. grown in the Botanical Garden of the Academy of Sciences of Uzbek SSR). A comparative analysis of the spectral figures showed that on passing from chloroform to pyridine the chemical shifts of the carbon atoms do not change appreciably either in the case of the 27-hydroxy compound (X) or in that of the diacetates (II) and (IX).

Consequently, it may be considered as established that the differences in the chemical shifts of the C-24, C-26, C-27, and C-28 carbon atoms in relation to those of withasteroids with a 25-CH_2OH group that have been mentioned above are due to the presence of the hydroxymethyl function at C-24 of the lactone ring of the new hydroxywithaphysanolide (I). In addition, the diamagnetic shifts of the C-24 signal observed on the acetylation of the 28-OH group and of the C-25 signal on the acetylation of the 27-OH function atmount to 7.1 and 3.6 ppm, respectively. In other words, the value of the β contribution of the acetoxy group depends substantially on its position in the lactone ring.

The facts given above on the assignment of the signals and the analysis of the values of the chemical shifts of the carbon atoms of the methyl groups, of the primary alcohol group, and also of the C-24 and C-25 signals of the lactone ring and of a number of characteristic carbon atoms of the steroid moiety in combination permit the conclusion that the primary hydroxy group in the hydroxywithaphysanolide (I) is attached to C-28. Consequently, compound (I) has the structure of 4β-14α,17β,20R,28-pentahydroxy-1-oxo-22R-witha-2,5,24-trienolide.

^{13}C NMR Spectra of Withasteroids from *Datura stramonium*

Having performed an analysis of the characteristics of the ^{13}C NMR spectra of the withasteroids, we may note that the CSs of the carbon atoms from C-1 to C-21 of withastramonolide (VIII) and of daturolactone B (XI) are respectively identical. This confirms the identity of the structures of the steroid moieties of their molecules. In the spectrum of compound (VIII) a singlet at 73.8 ppm belongs to C-5. The single α-orientated tertiary OH group is attached to this carbon atom. Doublets with the same CSs (56.4 ppm) must be assigned to the C-6 and C-7 carbon atoms bearing the epoxide ring. A doublet at 71.9 ppm is characteristic for C-12 bearing an α-orientated secondary OH group. If its orientation were β, this signal would be located in a weaker field (at 79-80 ppm [23]). A triplet at 30.4 ppm and a singlet at 43.9 ppm are assigned, respectively, to C-11 and C-13. In the spectrum of daturolactone A (XII), these signals undergo a substantial paramagnetic shift and are located at 39.0 and 57.9 ppm, which are due to the influence of the 12-oxo function. It can be seen from Table 1 that on passing from compounds (VIII) and (IX) to (XII) the CSs of the C-9 and C-14 carbon atoms also undergo a marked change. This obviously must be regarded as the consequence of the absence of the γ-effect of the 12-hydroxy group and not of the influence of a 12-oxo function, since the latter acts only slightly on the characteristics of the atoms under consideration [24].

In the ^{13}C NMR spectrum of withastramonolide (VIII) there are four quartet signals of methyl groups at 11.9, 14.9, 12.5, and 19.9 ppm, among which only the last changes its position on passing to daturolactone A (XII) (17.7 ppm). This is obviously due to the epoxidation of the Δ^{24}-double bond. We assign a quartet at 19.9 ppm to CH_3-28, and the remaining three signals are distributed between CH_3-18, -19, and -21. Furthermore, the signals of the hydroxymethyl carbon atom of the withastramonolide (VIII) and of withaferin A (X) (27-CH_2OH) are characterized by practically identical positions in the spectra (56.1 and 56.6 ppm, respectively), while in the case of the 28-hydroxywithaphysanolide (I) the resonance line of the carbon atom of the 28-CH_2OH group is located at 60.9 ppm (see Table 1). Consequently, these facts show with greater definiteness that the parimary alcohol group in withastramonolide (VIII) is located at C-27, and this agrees well with the structure of its molecule [16].

To confirm the corrections introduced into the structural formulas of withaphysanolide (III) and physalactone (VI) through a careful analysis of the characteristics of their ^{13}C spectra, we attempted to link (III) and (IV) chemically with 4β-hydroxywithanolide E (VII).

In an alkaline medium, methanol added to the Δ^2-double bond of 4-hydroxywithanolide E (VII) to form physalactone (VI). The reaction of withaphysanolide (III) with concentrated H_2O_2 formed 2,3-epoxywithaphysanolide (V). However, the action of perbenzoic acid on the withasteroid (III) led to the attachment of an epoxy group in the Δ^5-double bond, forming 4β-hydroxywithanolide E (VII).

These transformations showed that the withasteroids (III), (VI), and (VII) have related structures and differ by the substituents in rings A and B. Thus, withaphysanolide (III) has the structure of 4β,14α,17β,20R-tetrahydroxy-1-oxo-22R-witha-2,5,24-trienolide, and physalactone (VI) that of 4β,14α,17β,20R-tetrahydroxy-3β-methoxy-1-oxo-5β,6β-epoxy-22R-witha-2,24-dienolide.

EXPERIMENTAL

For thin-layer chromatography (TCL) we used type KSK silica gel containing 5% of gypsum. The following solvent systems were used: 1) chloroform—methanol (10:1); 2) hexane—acetone (1:1); 3) chloroform—benzene—methanol (5:5:1). The revealing agent was a saturated solution of $SbCl_3$ (with heating). UV spectra were taken on a Specord UV-VIS spectrophotometer, IR spectra on a UR-20 instrument, and mass spectra on a MKh-1303 spectrometer fitted with a system for the direct introduction of the sample into the ion source at an inlet temperature of 160°C, an emission current of 50 μA, and an ionizing voltage of 40 V. PMR spectra were taken on a XL-200 spectrometer (Varian) with 0—TMS, and a JNM-4H100 (JEOL) spectrometer; ^{13}C spectra were obtained on a CFT-20 instrument (Varian) with 0—TMS.

Isolation of 28-Hydroxywithaphysanolide (I). On continuing the washing of the column [1] with n-hexane—acetone (2:3) we obtained several individual substances in very small amounts. The most polar of them (TLC, system 2), after recrystallization from methanol, had mp 234°C. The yield of this substance on the weight of the air-dry raw material was 0.03%. 28-Hydroxywithaphysanolide: $C_{28}H_{38}O_8$, $\lambda_{max}^{C_2H_5OH}$: 212 nm (log ε 4.10); λ_{max}^{KBr} (cm^{-1}): 3400-3300 (1715, 1660). Mass spectrum, m/z (%): M^+ 502 (0, $^-$), 484 (6.5), 466 (20), 488 (10), 450 (6.5), 343 (13), 325 (27), 307 (53), 299 (5), 238 (83), 185 (100), 171 (33), 168 (35), 141 (40).

4,28-Di-O-acetyl-28-hydroxywithaphysanolide (II). 28-Hydroxywithaphysanolide (100 mg) was dissolved in 3 ml of dry pyridine and 3 ml of acetic anhydride. The reaction mixture was left to stand for a day and was then worked up in the usual way. After recrystallization from aqueous methanol, the diacetate $C_{32}H_{42}O_{10}$ had mp 204°C, $[\alpha]_D^{22}$ + 89.6 ± 3° (c 1.894, chloroform). ν_{max}^{KBr} (cm^{-1}): 3400, 1715, 1600, 1230, 1080. Mass spectrum, m/z (%): 568 (15), 526 (66), 508 (100), 466 (23), 448 (50), 385 (30), 341 (30), 325 (100), 299 (100), 227 (45), 183 (40). PMR spectrum (C_5D_5N, δ, ppm): 1.46 (3 H, s, CH_3-18); 1.56 (3 H, s, CH_3-19); 1.80 (3 H, s, CH_3-21); 2.06 (3 H, br.s., CH_3-27); 4.75 and 5.01 (1 H each, d, ^2J = 13.6 Hz, 2 H-28); 5.31 (1 H, q, ^3J = 13.3 and 3.0 Hz, 22-H); 6.77 (1 H, q, ^3J = 9.9 and 4.8 Hz, H-3); 6.11 (1 H, d, ^3J = 9.9 Hz, H-2); 5.91 (1 H, d, ^3J = 4.9 Hz, H-4); 6.20 (1 H, q, ^3J = 5.2 and 2.0 Hz, H-6).

Oxidation of the Diacetate (II) to the Lactone (XIII) and Androstan-2,5-diene-1,17-dione (XIV). The diacetate (II) (100 mg) was dissolved in 5 ml of acetone and, with ice cooling, the Jones reagent was added dropwise until the initial compound could no longer be detected on TLC (system 3). The reaction mixture was diluted with 10 ml of water and extracted with chloroform. The chloroform solution was washed with water, dried over Na_2SO_4, and evaporated. The residue consisted of 87 mg of a viscous oily substance which was separated preparatively on plates with a thin layer of silica gel in system 3. Two products were obtained. The first consisted of a yellowish oil (XIII) with the composition $C_{11}H_{14}O_5$. Mass spectrum, m/z (%): 183 (100), 149 (52), 140 (60), 123 (78), 43 (87). PMR spectrum ($CDCl_3$, δ, ppm): 1.86 and 1.93 (3 H each, methyl groups at a double bond); 2.54 (2 H, d, CH_2); 4.63 (1 H, tr, lactone proton); 2.30 (3 H, s, acetyl group).

The other product consisted of a crystalline substance (XIV) with the composition $C_{21}H_{26}O_5$, mp 140°C, $[\alpha]_D^{25}$ + 118 ± 3° (c 1.45, methanol), $\lambda_{max}^{C_2H_5OH}$ 210 nm (log ε 3.6); ν_{max}^{KBr} (cm^{-1}) 3450, 1725, 1675, 1670, 1245. Mass spectrum, m/z (%): M^+ 358 (5), 340 (4), 298 (100) M — CH_3COOH 280 (90), 265 (25), 233 (48), 209 (25), 171 (90). PMR spectrum (C_5D_5N, δ, ppm): 1.03 (3 H, s, CH_3-18); 1.53 (3 H, s, CH_3-19); 2.12 (3 H, s, OAc); 6.66 (1 H, q, J = 9.2 and 5.2 Hz, H-3); 6.02 (1 H, d, J = 9.2 Hz, H-2); 5.72 (1 H, d, J = 5.2 Hz, H-4); 6.15 (1 H, br.d, H-6).

A mixed melting point of the androstadiene (XIV) obtained from the diacetate of 28-hydroxywithaphysanolide (II) and from withaphysanolide acetate gave no depression of the melting point. On TLC in systems 1-3, the compounds were identical.

Physalactone (V) from 4β-Hydroxywithanolide E (VII). 4β-Hydroxywithanolide E (VII) (30 mg) was dissolved in 20 ml of methanol, and 2-3 drops of a 2% solution of K_2CO_3 in methanol was added. Monitoring was carried out with the aid of TLC in system 3. After 2 hours at room temperature, the initial compound had disappeared completely. The reaction mixture was diluted with 50 ml of water and extracted with chloroform. The chloroform solution was washed with water, dried over Na_2SO_4, and evaporated. Chromatography on silica gel in system 2 gave 25 mg of amorphous physalactone (VI), with the composition $C_{29}H_{42}O_9$, $[\alpha]_D^{22}$ −4.0 ± 2° (c 2.0, methanol). $\lambda_{max}^{C_2H_5OH}$ 228 nm (log ε 3.94). Mass spectrum, m/z (%): M^+ 516 (1), 484 (2), 466 (10), 448 (10), 430 (2.5), 391 (10), 363 (6), 359 (5), 347 (5), 341 (4), 326 (3.5), 213 (14), 169 (30), 152 (74), 125 (100). PMR spectrum ($CDCl_3$, δ, ppm): 1.06 (3 H, s, CH_3-18); 1.30

(3 H, s, CH_3-19); 1.45 (3 H, s, CH_3-21); 1.90 and 1.97 (3 H each, br. singlets, C-27 and -28); 3.36 (3 H, s, OCH_3); 4.87 (1 H, tr., 22-H).

4β-Hydroxywithanolide E (VII) from Withaphysanolide (III). Withaphysanolide (100 mg) was dissolved in 20 ml of chloroform and 50 ml of benzene. After the addition of 20 ml of a chloroform solution of perbenzoic acid [25] the mixture was left at 5°C for 20 h. Then it was washed with a 2% solution of $NaHCO_3$ and with water, was dried over Na_2SO_4, and was evaporated in vacuum. The residue was chromatographed on silica gel and eluted with system 2. Fractions containing product (VII) were combined and evaporated, and the residue was recrystallized from ethyl acetate. This gave 20 mg of 4β-hydroxywithanolide E with mp 228-230°C, $[\alpha]_D^{25}$ + 85 ± 3° (c 1.30, ethanol), $\lambda_{max}^{C_2H_5OH}$ 220 nm (log ε 4.10). A mixed sample with 4β-hydroxywithanolide E isolated directly from a plant showed no melting depression.

2,3-Epoxywithaphysanolide (V) from Withaphysanolide (III). The withasteroid (III) (30 mg) was dissolved in 10 ml of isopropanol, and, at room temperature, 15 ml of 35% H_2O_2 and one drop of a 10% solution of KOH were added. After 20 h, 50 ml of water was added to the reaction mixture, and it was neutralized with 5% HCl to pH 7 and was extracted three times with 20-ml portions of chloroform. The chloroform extract was washed with water, dried over Na_2SO_4, and evaporated in vacuum. When the residue was treated with 1 ml of methanol, a crystalline deposit of 2,3-epoxywithaphysanolide (V) separated out. PMR spectrum (C_5D_5N, δ, ppm): 1.41 (3 H, s, CH_3-18); 1.70 (3 H, s, CH_3-19); 1.78 (3 H, s, CH_3-21); 1.79 and 1.97 (3 H each, singlets, CH_3-27 and CH_3-28); 3.81 (1 H, d, J = 4.6 Hz, H-2); 3.98 (1 H, tr., ^3J = 8.0 Hz, H-3); 5.03 (1 H, d, ^3J = 3.4 Hz, H-4); 5.25 (1 H, q, ^3J = 13.1 and 3.4 Hz, H-22); 5.78 (H-6).

SUMMARY

1. The leaves of *Physalis viscosa* L. (family Solanaceae) have yielded a new withasteroid — 28-hydroxywithaphysanolide, having the structure of 4β,14α,17β,20R,28-pentahydroxy-1-oxo-22R-witha-2,5,24-trienolide.

2. The ^{13}C NMR spectra of compounds of the 14β-hydroxywithasteroid series from *Physalis viscosa*, of the 5α-hydroxywithasteroids from *Datura stramonium* L., and some of their derivatives have been studied. An assignment of their signals has been made on the basis of a mutual comparison of the characteristics of the spectra of the compounds investigated and of other related compounds. The characteristics of the most informative carbon atoms in the ^{13}C NMR spectra have been discussed in the light of the structural and stereochemical features of the compounds investigated.

3. Features of the change in the value of the chemical shifts of the carbon atoms of the lactone rings of the withasteroids as functions of the positions of the hydroxymethyl group have been elucidated.

4. On the basis of an analysis of the characteristics of the ^{13}C NMR spectra, corrections have been made in the structural formulas of physalactone, which has been shown to be 4β,14α,17β,20R-tetrahydroxy-3β-methoxy-1-oxo-5β,6β-opoxy-22R-witha-24-enolide, and of withaphysanolide, which has been shown to be 4β,14α,17β,20R-tetrahydroxy-1-oxo-22R-witha-2,5,24-trienolide. The conversion of withaphysanolide into 4β-hydroxywithanolide E and of the latter into physalactone has confirmed the common basic structures of these withasteroids.

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TRITERPENE SAPONINS FROM *Thalictrum minus*.

III. THE STRUCTURE OF THALICOGENIN

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The periodate oxidation with subsequent alkaline degradation of the products of the oxidation of the predominant saponin isolated from *Thalictrum minus* has yielded the native genin, which has been called thalicogenin. The structure of thalicogenin as 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene has been established on the basis of chemical transformations and by spectral methods.

It has been reported previously that the acid hydrolysis of the predominating saponin isolated from *Thalictrum minus* (low meadow rue) gives a mixture of artefacts with a predominance of a compound for which the structure of 3β,16β,29-trihydroxy-22,25-epoxylanost-9(11)-ene (II) has been established [1-3]. In the present communication we describe the isolation of the native genin (I) and the determination of its structure.

The genin (I), which has been called thalicogenin, was obtained by the periodate oxidation of the main saponin of the plant under investigation followed by alkaline degradation of the oxidation products. According to the results of elementary analysis and its molecular mass (M^+ 474), thalicogenin corresponds to the formula $C_{30}H_{50}O_4$. The PMR spectrum has the signals of six methyl groups, and the mass spectrum (scheme 1) shows fragments with m/z (%): 329 (100) and 109 (17.3), which gives grounds for assuming that the compound isolated is a tetracyclic triterpenoid with an aliphatic side chain [4].

The structure of the side chain of thalicogenin was established in the following way. It followed from the PMR spectrum that compound (I) has a secondary methyl group (1.23 ppm,

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