WITHASTEROIDS OF Physalis.

X. CIRCULAR DICHROISM OF WITHASTEROIDS FROM PLANTS OF THE GENUS Physalis

G. P. Moiseeva, O. E. Vasina,
and A. K. Abubakirov

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The circular dichroism method has been used to establish the stereochemical features of the structure of the withasteroids. The 22S-configuration of the asymmetric center has been confirmed for physangulide. The 22R-configuration has been confirmed for 14α-hydroxyixocarpanolide, vamonolide, ixocarpanolide, 24,-25-epoxywithanolide D, and withanolide T. On the basis of changes in the circular dichroism spectra in acid and alkaline media it is suggested that in compounds having a 4β-hydroxy-1-oxo-5β,6β-epoxy-2,3-ene grouping the $\Delta^{2,3}$ bond disappears, while in compounds having a 5α-hydroxy-1-oxo-6α,7α-epoxy-2,3-ene grouping dehydration takes place at C-4—C-5. These characteristics can be used in the identification of the withasteroids. The isolation of withanolide T from the leaves of *Physalis angulata* L. is described and its spectral characteristics are given.

We have used the method of circular dichroism (CD) for establishing the stereochemical features of withasteroid structures.

In the withasteroids, a δ-lactone ring is located in the side chain [1], which makes it conformationally mobile and capable of adopting the conformation of a distorted boat or a distorted chair, while, depending on the pH of the medium, conformational inversion is also possible. Six withasteroids isolated from plants of the genus *Physalis* have been investigated. For comparison we took daturalactone and withastramonolide, obtained from *Datura stramonium*.

A Cotton effect (CE), due to a $n \rightarrow \pi^*$ transition of the carbonyl of the lactone group and giving information relative to its stereochemistry appears in the CD spectra of withanolides containing a saturated δ-lactone ring (14α-hydroxyixocarpanolide (I) [2], vamonolide (II) [3], and ixocarpanolide (III) [4]) in the 210-220 mn region, while for those containing an epoxylactone ring (24,25-epoxywithanolide D (IV) [2] and daturalactone (VI) [13]) it appears in the 230-240 nm region and for withanolides with an α,β-saturated lactone ring (withastramanolide (VII) [12] and withanolide T (VIII)) in the 250-260 nm region. In the position and sign of the lactone Cotton effect, physangulide (V) differs from other saturated lactones ((I-III), Table 1).

The sign of the Cotton effect is determined by the chirality of the lactone group due to the asymmetry of the neighboring carbon atom and the conformation of the ring. If the C-20—C-22 bond is equatorial, it has been established for saturated δ-lactones that negative and positive Cotton effects correspond to the half-boat and half-chair conformations, respectively [6]. Since in the CD spectra of compounds (I-III) a negative (—) CE is observed in the 210-220 nm region, in these withasteroids, the lactone ring has the half-boat conformation with the diequatorial arrangement of the methyl groups. A structure with the axial-equatorial arrangement of the methyl groups at C-25 and C-24, respectively, is less preferred because of interaction of the "flagstaff" type.

As can be seen from Table 1 (compare compounds (I) and (II)), in an alkaline medium an inversion of the sign of the lactonic CE takes place which is connected with the transformation of the half-boat into the half-chair conformation. The equatorial methyl group at

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TABLE 1. Characteristics of the CD of Some Withasteroids in Neutral, Acid, and Alkaline Media

Compound	CH_3OH		$CH_3OH+HCl$		$CH_3OH+KOH$	
	λ_{max}, nm	Δε	λ_{max}, nm	Δε	λ_{max}, nm	Δε
I	337	−1,96	338	−2,10	386	+1,50
					325	−2,64
					227	+1,36
II	216	−1,70	217	−0,77	222	+1,53
	338	−2,04	338	−2,40	386	+0,92
					320	−3,48
	240	+0,30			238	+0,80
	215	−1,53	218	−0,90	228	+1,21
					222	+1,68
III	338	−2.39				
	241	+0,29				
	209	−1,56				
IV	345	+1.76				
	290	−0,23	288	−3,24	288	−5,23
	239	−1,95	229	+6,92	228	+1,53
	212	+13,7				
V	288	−4,94	288	−3,33	288	−5,02
	228	+3,64	230	+6.89	228	+3,92
VI	337	−1,97	337	−1,90	385	+2,42
					315	−6,18
	260	+0,40	260	+0,40		
	236	−2,54	236	−2,20	226	+5,03
	210	+8,34	210	+8,34		
VII	337	−1,90	337	−1,90		
	253	+4,00	253	+5,07		
	210	+11,5	210	+12,40		
VIII	337	22,34	337	−2,34	385	+0,86
	250	+5,00	259	+5,80	315	−4,28
	210	+9,80	215	+10,6	242	+4,90

C-25 becomes axial and is easily epimerized [5]. The change in the conformation of the lactone ring in compounds (I) and (II) in an alkaline medium is confirmed by the bathochromic shift of the lactone band to 222-225 nm [7]. It must be mentioned that when an alcoholic solution is left to stand for two days, more profound structural changes take place and the opening of the lactone ring is also possible. In the CD spectrum, a (+)-CE appears at 250 nm (Δε = +2.58) and a (−)-CE at 220 nm (Δε = −2.28).

For unsaturated δ-lactones, x-ray-structural analysis has established that only one carbon atom (C-22) the configuration of which is determined by the conformation of the lactone ring is displaced by 0.6-0.7 A from the plane containing the other carbon atoms [7].

(+) I

(−) II

For withanolides with a lactone ring in conformation (I) and the 22R-configuration, the Cotton effect in the 250-260 nm region should be positive, while for conformation (II) it should be negative [8, 9]. In the CD spectrum of withanolide T (VIII) (which we have isolated from Physalis angulata L., and have detected for the first time in Withania somnifera [10, 11]), and for withastromonolide (VIII) (isolated from Datura stramonium [12]) a (+)-CE is present in the 250 nm region, i.e., the lactone ring has conformation (I) and the 22R-configuration.

If in place of a double bond there is an epoxy group in the lactone ring, then the link between the chiral chromophore and the sign of the CE is the opposite, just as for a carbonyl chromophore [13]. Such an inverse dependence is observed, in particular, for daturalactone (VI). Consequently, the (−)-CE in the 240 nm region in the CD spectrum of 24,25-epoxywithanolide D correlates with the 22R-configuration. This configuration is confirmed by the values of the spin-spin coupling constants (SSCCs) in the PMR spectrum [2].

Withasteroid (V) (physangulide), has a saturated six-membered ring with a 24,25-diol group [5]. As can be seen from Table 1, according to the parameters of the lactonic Cotton effect, physangulide differs from the saturated lactones (I-III) discussed previously.

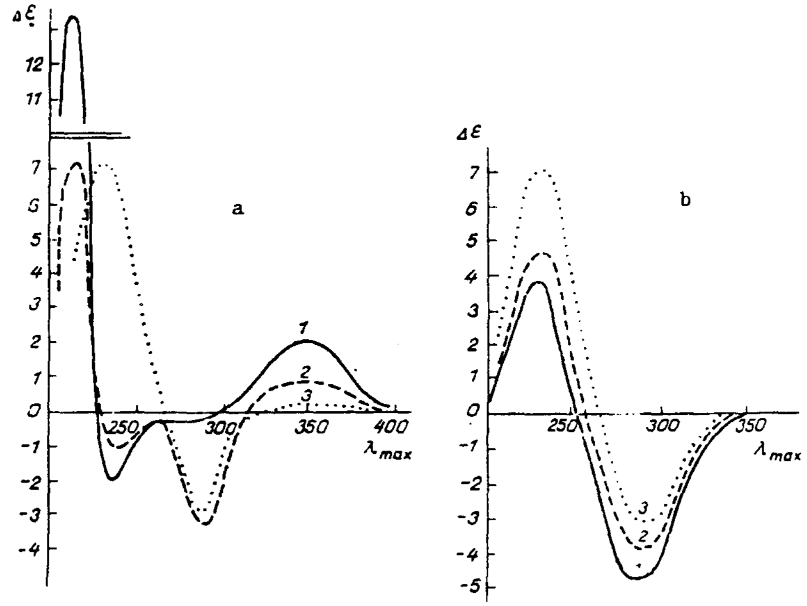
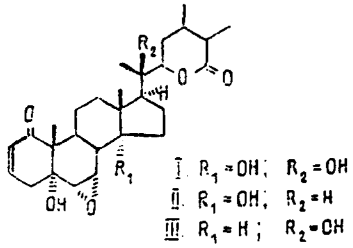
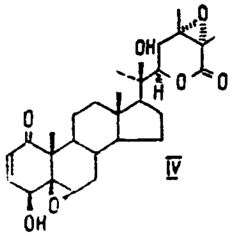


Fig. 1. CD spectra of 24,25-epoxywithanolide D (a) and of physangulide (b): 1) in methanol; 2) in methanol + HCl (after an hour); 3) in methanol + HCl (after a day).





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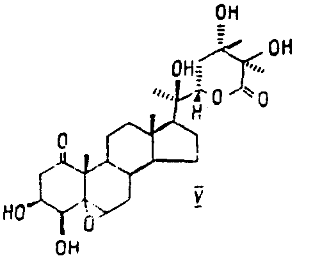

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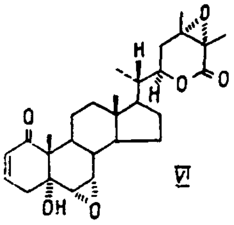
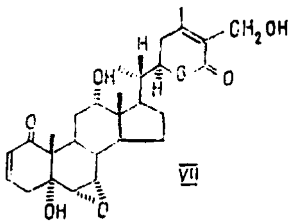
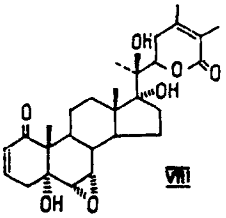


VI



VII



VIII

Since, in the PMR spectrum of this compound, the SSCC of the proton at C-22 unambiguously shows the 22S-configuration, the lactone ring apparently has the half-chair conformation. Such a structure is more stable with the axial orientation of the C-12—C-22 bond. The stability of this conformation is confirmed by the absence of an inversion of the sign of the CE at 228 nm in acid and alkaline media. Furthermore, the spectrum of compound (IV) in an acid medium shows the same CE at 228 nm. In an alkaline medium, compounds (IV) and (VI) have Cotton effects in the 226-228 nm region with a (+) sign, differing from the CE of physangulide (V) only in intensity.

It is known for the case of physapubescin [14] that the epoxide ring opens in an acid medium. In our experiment, a day after the acidification of methanolic solutions of 24,25-epoxywithanolide D (IV) and physangulide (V) their CD spectra became identical (Fig. 1).

In the CD spectra of the withanolides, Cotton effects due to the $n \rightarrow \pi^*$ transitions of the conjugated carbonyls of rings A were observed in the 340 nm region. The signs of these Cotton effects show the trans-linkage of rings A and B in withasteroids (I-III) and (VI-VIII) and the cis-linkage in compounds (IV) and (V) [15]. Depending on the position of a hydroxy group relative to the conjugated carbonyl group in ring A, different characteristic changes in acid and alkaline media are observed.

In the CD spectrum of 24,25-epoxywithanolide D (IV) (hydroxy group at C-4) on acidification and alkalinization the CE at 340 nm disappears and a CE characteristic for a saturated carbonyl group appears in the 290 nm region. In an alkaline medium, the changes observed are due to the ready formation of a methanol adduct at C-3 [16]. In each of compounds (I-III) and (VI-VIII) a hydroxy group is located at C-5. On alkalinization, CEs appear in the 385 nm region in the CD spectra of these compounds (see Table 1). We explain the shift by an increase in the length of the chain of conjugation through the formation of a $\Delta^{4,5}$ bond as the result of dehydration of the hydroxy group at C-5.

EXPERIMENTAL

CD spectra were recorded on a JASCO J-20 spectropolarimeter. The concentration of the solutions was 1 mg/ml, and the cell thicknesses 0.1, 0.05, 0.02, and 0.01 cm. Methanol was used as solvent. For acidification, 0.02 ml of HCl (the concentrated acid) was added to 3 ml of a methanolic solution. Alkaline solutions were obtained by dissolving a weighed amount of withasteroid in a 0.5% alcoholic solution of potassium hydroxide.

Isolation of Withanolide T. The epigeal part of *Physalis angulata* L. (7 kg) was extracted with hot water. The aqueous solution, after cooling, was extracted with chloroform. The solvent was evaporated off. This gave 37.4 g of material containing withasteroids. The extract was fractionated on a column of type KSK silica gel in the chloroform–acetone (5:1) system. A series of fractions was obtained from which several withasteroids were isolated by rechromatography in various systems [2, 3, 5]. The fractions containing withanoid T were combined and rechromatographed on a column in the hexane–acetone (1:1) system, and then the enriched fraction was evaporated and the residue was recrystallized from methanol. This gave 0.185 g of compound (VIII). On the basis of spectral characteristics (mass spectrum, PMR spectrum), we arrived at a structure identical with that of withanolide T [10, 11].

Withanolide T (VIII), $C_{28}H_{38}O_7$, mp 233-236°C (from methanol) $\lambda_{\max}^{C_2H_5OH}$: 227 nm ($\varepsilon$ = 23000); $\nu_{\max}^{KBr}$, cm^{-1}: 3590–3430, 2950, 1705, 1687; mass spectrum, m/z (%): 486 M^+(0.39), 399(28.8), 325(53.0), 265(71.2), 171(98.5), 125(100).

PMR spectrum (100 MHz, DPy, 0–TMS, δ scale), 1.26 s (3H, 19-CH_3), 1.38 s (3H, 21-CH_3), 1.45 s (3H, 18-CH_3), 1.58 s and 1.87 s (each 3H, 27 and 28-CH_3), 3.08 d (1H, H-6), 3.48 dd (1H, H-7), 4.41 dd (1H, H-22), 5.99 dd (1H, H-2), 6.56 ddd (1H, H-3).

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PRODUCTION OF SORBENTS FOR REVERSED-PHASE CHROMATOGRAPHY BY THE ALKYLATION OF THE SURFACE OF SILICA GEL WITH ALCOHOLS

V. I. Grishkovets, A. A. Poloiko,
and V. Ya. Chirva

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A procedure is proposed for alkylating the surface of silica gel with heptyl, decyl, and dodecyl alcohols with the aim of obtaining sorbents for reversed-phase chromatography. The efficacy of the use of the sorbents obtained for the purification and separation of triterpene glycosides has been shown.

The chemical modification of the surface of silica gels by various organic and inorganic substances permits the purposeful production of sorbents with new properties [1]. In liquid reversed-phase chromatography, sorbents with grafted-on alkyl groups (1-22 methylene units) are widely used, while more than half the sorbents marketed abroad have been modified by treating the surface of silica gel with octadecyldimethylchlorosilane − the so-called ODS sorbents [2]. They are distinguished by a high hydrolytic stability of the Si—O—Si—C bond of the modifying agent with the support.

Supports with grafted-on alkoxy groups are also fully suitable for preparative liquid chromatography even though the Si—O—C bond is not as resistant to hydrolysis, particularly at elevated temperatures. The alkylation of activated silica gel with (C_1-C_8) alcohols was first described by Deuel et al. [3]. However, the degrees of coverage that they achieved were low in comparison with modern ODS sorbents, and the chromatographic characteristics of the products were not described.

We propose a procedure for alkylating the surface of silica gel with alcohols in which a high degree of coverage of the surface by alkoxy groups and good chromatographic characteristics in the separation of triterpene glycosides are achieved.

The surface of silica gel after hydroxylation and drying in vacuum with the aim of eliminating physically adsorbed water was activated by converting the surface silanol Si—OH groups into Si—Cl groups by treatment with thionyl chloride in benzene. Then the chlorinated silica gel was treated with the modifying alcohol in the presence of pyridine in boiling benzene.

Table 1 gives the results of the analysis of silica gel L (Czechoslovakia) alkylated with heptyl, decyl, and dodecyl alcohols. The percentages of alkyl groups calculated on the basis of the results of microanalysis for carbon and of thermogravimetric results practically coincided. The densities of grafting calculated in the light of the specific surface of silica gel L, 600 m^2/g [2], were comparable with results of investigations in which alkyldimethylchlorosilanes were used as modifying agents [4, 5].

M. V. Frunze Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 376-378, May-June, 1990. Original article submitted June 7, 1989.

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