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CHEMISTRY AND GENETICS OF WITHANOLIDES IN *WITHANIA SOMNIFERA* HYBRIDS

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Key Word Index—*Withania somnifera*; Solanaceae; withanolides; steroidal lactones; hybrid plants and genetics.

Abstract—Hybrid plants of *Withania somnifera* from cross-pollinations of either chemotypes II or III (Israel) and Indian I (Delhi) have been examined. From both hybrids, 14β-hydroxywithanone (5α,14β,17α-trihydroxy-6α,7α-epoxy-1-oxo-22*R*-witha-2,24-dienolide) has been identified. This compound is the first example of a 14β-substitution among withanolides. From the second hybrid three additional new compounds were characterized: a 2,4,6-trien-1-one ($14\alpha,20\alpha_F$-dihydroxy-1-oxo-22*R*-witha-2,4,6,24-tetraenolide), a 14α-hydroxywithanone (5α,14α,17α-trihydroxy-6α,7α-epoxy-1-oxo-22*R*-witha-2,24-dienolide) and a 6β,7β-epoxywithanone (5α,14α,17α-trihydroxy-6β,7β-epoxy-1-oxo-22*R*-witha-2,24-dienolide). An analysis of the inheritance characteristics of various substituents on the withanolide skeleton was based on the occurrence in per cent of each substituent in relation to the total withanolide content in the hybrid plants and their respective parents.

INTRODUCTION

It has been shown earlier that the hybrid plants of *Withania somnifera* L. (Dun.) chemotype III (Israel) by Indian I (Delhi) elaborated withanolides with new nuclear substitutions according to the combined genetic inheritance. Thus, two new compounds, withanolides T and U, were isolated and described [1]. A more detailed examination of these hybrid plants led to the isolation of five additional compounds, two of which were reported in earlier communications: one dealing with the structural analysis of a 6α,5β-chlorohydrin **5** [2], and the other with the X-ray structure analysis of 14β-hydroxywithanone (**3**) [3]. We now discuss the latter compound in more detail, together with the other three withanolides, namely, the 2,4,6-trien-1-one (**1**), 14α-hydroxywithanone (**2**) and its unusual isomer, the 6β,7β-epoxide (**4**). New hybrid plants resulting from *Withania somnifera* chemotype II (Israel) by Indian I (Delhi) were also analysed, and a comparative study of the withanolide constituents of these hybrids were made, leading to some interesting observations of the genetics.

RESULTS AND DISCUSSION

Compound **1** ($C_{28}H_{36}O_5$) was found to be a 2,4,6-trien-1-one and its structure was established on the basis of spectral data. Its UV absorptions at 352 and 228 nm (ε 3500 and 10 000) are good evidence for such a system in rings A and B and for the usual unsaturated δ-lactone system of the side-chain. Complete structural information was obtained from the ^{1}H NMR spectrum, Table 1. The positions of the 2,3,4,6- and 7-protons were assigned by appropriate sequential double irradiations. Similar protons were measured and observed for compound **6**, a reaction product obtained from withanone* (**7**) [5], for which the low field signals were identical. Compound **1** has no acetylable hydroxyl groups, but a tertiary hydroxyl could be located at C-20 in view of the shape of the signal of the 22-H and, moreover, its position also indicated the absence of a C-17-OH. According to the molecular ion in the mass spectrum, an additional oxygen had to be accounted for. This was placed as a 14α-hydroxyl on the basis of the ^{1}H NMR spectral data for the 8-H (δ 2.74, *br d*, J = 12 Hz, changing to a sharp *d* upon irradiation at the resonance of 7-H) which showed that only one proton is present on a vicinal carbon (C-9) in a *trans*-diaxial relationship, (Table 1).

Compound **2** ($C_{28}H_{38}O_7$) was assigned the structure of 14α-hydroxywithanone, based on ^{1}H NMR spectral considerations. The spectrum was indeed very close to that of withanone (**7**) [5]. The only remarkable difference was for the 18-Me resonance which in the former was downfield by 0.11 ppm with respect to the corresponding signal in the spectrum of **7**. Furthermore, an additional oxygen had to be present in **2** as shown by the molecular ion at *m*/*z* 486. Accordingly, a hydroxyl group was placed at C-14 and given the α-orientation, since the signal position of the 18-Me was not affected when the ^{1}H NMR spectrum was taken in C_5D_5N. This was also true for 21-Me. Hence the C-17 hydroxyl is also α-oriented, as expected. Attempts to make an acetonide with the C-14 and C-17 hydroxyl groups under acidic conditions resulted in a facile elimination of the C-14 hydroxyl, which was expected [6], producing compound **8** (see below).

Compound **3** ($C_{28}H_{38}O_7$) was assigned the structure 14β-hydroxywithanone by an X-ray crystal analysis which was the subject of an earlier communication [3] (see Fig. 1 for a stereoscopic view). This structure with a 14β hydroxyl group on a withanone skeleton, accounted for the anomalous ^{1}H NMR low field positions of 18-Me, 20-H and 7-H, compared to those of withanone (**7**). The orientation of this unusual 14β hydroxyl group is axial towards ring C. As seen in Fig. 1, the strain imposed by the *cis*-fusion of rings C/D results in the 18-Me group

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* Withanone is the name given to a compound isolated from *Withania somnifera* collected in the area of Delhi [4], and first identified as compound IV in ref. [5]. This name is now adopted.

Table 1. ^{1}H NMR spectral signals of relevant protons in withanolides

Compound								Me groups					Other
	2-H	3-H	4-H$_{ax.}$	4-H$_{eq.}$	6-H	7-H	22-H	18	19	21	27	28	signals
1	5.97 (*d*, 9.3)	6.99 (*dd*, 9.4, 6.5)	5.97 (*d*, 5.3)		5.86 (*dd*, 9.4, 1.8)	6.30 (*dd*, 10.0, 2.9)	4.22 (*dd*, 13.2, 3.8)	1.15	1.30	1.20	1.89	1.95	2.74 (*br d*, 12.0), 8-H
2	5.85 (*dd*, 10.0, 2.3)	6.64 (*ddd*, 10.0, 5.0, 2.3)	2.68 (*br d*, 18.7)	2.55 (*dd*, 18.7, 5.3)	2.98 (*d*, 4.1)	3.32 (*dd*, 3.8, 1.6)	4.70 (*ddd*, 10.0, 6.8, 2.6)	0.97	1.20	1.08 (*d*, 6.3)	1.88	1.94	2.40 (*m*), 20-H
					[3.05]	[3.37]	[4.78]	[0.92]	[1.27]	[1.17]	[1.68]	[1.94]	
3	5.86 (*dd*, 10.6, 2.6)	6.61 (*ddd*, 10.5, 5.2, 2.4)	2.67 (*br d*, 19.0)	2.59 (*dd*, 19.0, 5.0)	3.04 (*d*, 3.8)	3.78 (*dd*, 3.8, 1.6)	4.71 (*ddd*, 12.9, 4.0, 4.0)	1.16	1.18	1.05 (*d*, 6.3)	1.89	1.95	2.67 (*m*), 20-H
					[3.07]	[3.45]	[4.78]	[0.92]	[1.27]	[1.13]	[1.68]	[1.90]	
4	5.83 (*d*, 9.4)	6.61 (*m*)	3.19 (*br d*, 19.5)	2.15 (*dd*, 19.2, 5.0)	3.72 (*d*, 4.0)	3.79 (*m*)	4.64 (*m*)	0.86	1.32	1.07 (*d*, 6.3)	1.86	1.96	
			[3.64]		[4.19]	[4.32]	[4.82]	[0.93]	[1.69]	[1.28]	[1.74]	[1.95]	
7	5.81 (*dd*, 10.0, 2.3)	6.60 (*ddd*, 10.0, 5.0, 2.3)	2.69 (*br d*, 18.7)	2.53 (*dd*, 18.7) 5.3)	3.06 (*d*, 4.1)	3.34 (*dd*, 3.8, 1.6)	4.64 (*ddd*, 12.3, 4.6, 4.0)	0.86	1.18	1.03 (*d*, 6.3)	1.88	1.93	2.41 (*m*), 20-H
					[3.08]	[3.30]	[4.82]	[0.84]	[1.25]	[1.20]	[1.72]	[1.94]	
8	5.88 (*dd*, 10.0, 2.4)	6.61 (*ddd*, 10.0, 5.0, 2.4)	2.71 (*br d*, 19.0)	2.55 (*dd*, 19.0, 5.0)	3.11 (*d*, 3.8)	3.58 (*dd*, 3.8, 3.1)	4.59 (*ddd*, 12.9, 4.0, 4.0)	1.06	1.21	1.05 (*d*, 6.5)	1.89	1.95	5.47 (*br s*), 15-H

Chemical shifts (at 270 MHz) are in δ units; coupling constants (in Hz) are in parentheses; data measured in C_5D_5N are in square brackets.

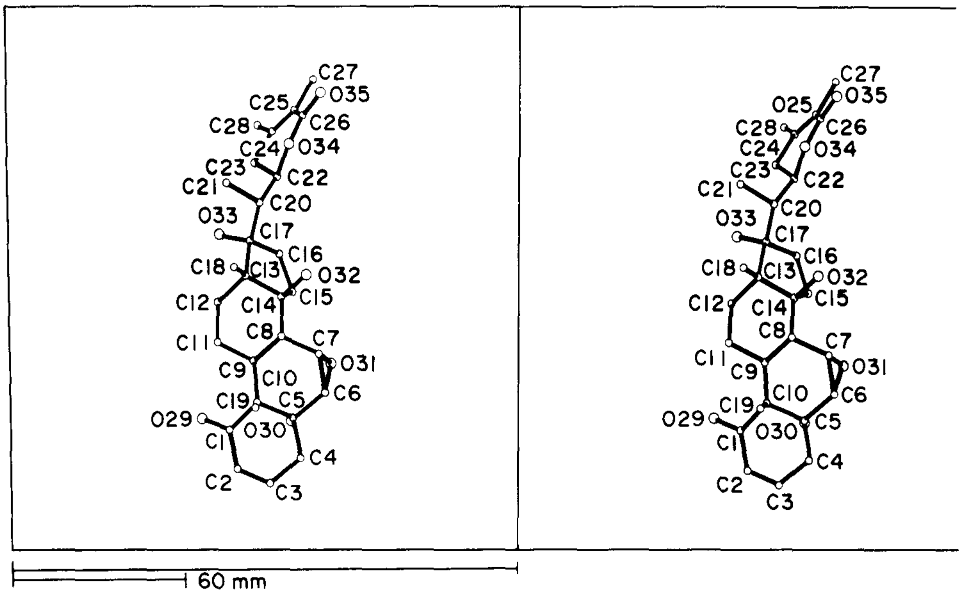


Fig. 1. Stereoscopic view of 14β-hydroxywithanone **3**.

and the 7β-H being in the deshielding influence of the 14β-hydroxyl ($\Delta = 0.31$ and 0.45 ppm) with respect to the corresponding protons in withanone (**7**). It can also be seen that the 20α_F-H orients itself towards this OH group and is, therefore, affected. The relative conformation of the rest of the molecule is as in the other withanolides. Thus, ring A has a quasi-boat (or envelope shape) conformation, B is a strained chair, C a normal chair and D has an envelope shape with C-14 being out-of-plane. Compound **3** was found to be very sensitive to acid, producing the Δ^{14}-derivative **8**, which was also obtained from 14α-hydroxywithanone (**2**). In this compound, the newly formed vinylic 15-H resonates in a position similar to that of the other Δ^{14}-compounds [6]. However, the 15-H signal value is slightly downfield (by 0.19 ppm), due to the vicinity of the epoxide oxygen. Conversely, the location of 7-H is also downfield (by 0.25 ppm), in this case due to the influence of the π-system of the Δ^{14} bond.

Compound **4** ($C_{28}H_{38}O_7$) was established as 6β,7β-epoxy-14α-hydroxywithanone. The ^{1}H NMR spectrum of this compound is similar to that of withanone (**7**). However, some exceptions could be recognized; the two epoxide protons reciprocally coupled appear at low field at δ 3.72 (*d*, $J = 4$ Hz) and 3.79 (*m*) which is due to their α-orientation and the influence of the 5α- and 14α-OH groups, respectively. The β-epoxide deshields the 19-Me by 0.12 ppm relative to that of compound **7**, in which the epoxide is α-oriented and away from this Me group. Furthermore, a sizeable pyridine-induced shift is observed for this Me group in **4** (Δ, 0.37 ppm), compared to **7** (Δ, 0.07 ppm). The same is also true for the two epoxide protons (Δ, 0.47 and 0.53 ppm for 6α-H and 7α-H respectively), which are strongly deshielded compared to those of withanone (**7**) (Δ, 0.02 and -0.04 for 6β-H and 7β-H respectively).

The ^{13}C NMR spectral assignments of all carbons in compounds **2** and **3** are presented in Table 2, together with those of withanone (**7**) which are given for comparison. They are in agreement with their assigned structures and with similar assignments described for several withanolides [7].

Biogenetic considerations

It has been shown earlier that *Withania somnifera* occurs as various chemotypes and is a good model for the study of chemogenetics [8]. When hybrids were analysed, new nuclear substitutions were observed according to the combined genetic inheritance from both parents [1,9]. During a re-examination of the hybrids from chemotypes III (Israel) by Indian I (Delhi), the additional compounds **1**–**5** were obtained, which can fit well into the biogenetic scheme previously presented [1,10]. The possible formation of these compounds is schematically presented separately for rings A/B (Scheme 1) and C/D (Scheme 2).

The origin of the chlorinated compound **5** has been discussed elsewhere [2]. The formation of the 6,7α- and 6,7β-epoxides originated from the intermediate **10**, to the predominant advantage of the former, which may be due to the directing effect of the 5α-hydroxyl. Comparing this situation with the 5,6-epoxywithanolides, the β-orientation is favoured exclusively, even in the absence of a 4β-hydroxyl (cf. withanolide E [11]). However, in the physalins, the epoxide may also have an α-orientation, though in minor quantities [12].

The isolation of a withanolide with a 14β-hydroxyl, which is unique so far, is accounted for in Scheme 2. Accordingly, the initial hydroxylation at C-14 takes place with retention of configuration, followed by an inversion, possibly through a C-14 carbocation. The 14β-hydroxysteroids are usually members of the cardenolide and bufanolide groups. Because the C-14 epimers of these compounds are not known, the occurrence of a 14β-hydroxywithanolide among compounds with a 14α-configuration is of special interest.

We have previously discussed an interpretation of inheritance characteristics of the hydroxyl and epoxide group substitutions at different positions based on their being either dominant or recessive during hybridization. For example, the 4β-hydroxyl and 5,6β-epoxide groups as well as the 20-hydroxyl are formed through characters which are dominant, whereas the 14α-hydroxyl and 27-hydroxyl are derived through recessive ones [8]. The

Table 2. ^{13}C NMR spectra of withanolides in DMSO-d_6

Carbon	2	3	7
1	203.5	203.6	203.4
2	127.8	127.8	128.1
3	141.1	141.2	140.9
4	*	*	36.9
5	72.9	72.6	72.9
6	52.7^a	53.1^a	55.1^a
7	53.6^a	53.7^a	55.2^a
8	*	*	35.7
9	25.3	30.8	34.5
10	49.8	50.6	50.4
11	20.1	21.5	21.1
12	33.5^b	34.3	32.4
13	50.6	52.4	48.2
14	84.4	83.5	45.1
15	28.1	31.6	22.5
16	*	*	36.3
17	86.0	83.1	83.1
18	18.8	16.8	14.2
19	13.8	13.8	14.7
20	43.4	41.6	42.9
21	9.7	10.0	9.4
22	78.7	78.4	78.5
23	33.1^b	32.9	32.0
24	151.3	151.9	150.8
25	119.8	119.9	120.1
26	166.1	166.1	166.0
27	12.4	12.1	12.0
28	19.8	20.1	20.1

*Signal superimposing with that of solvent.

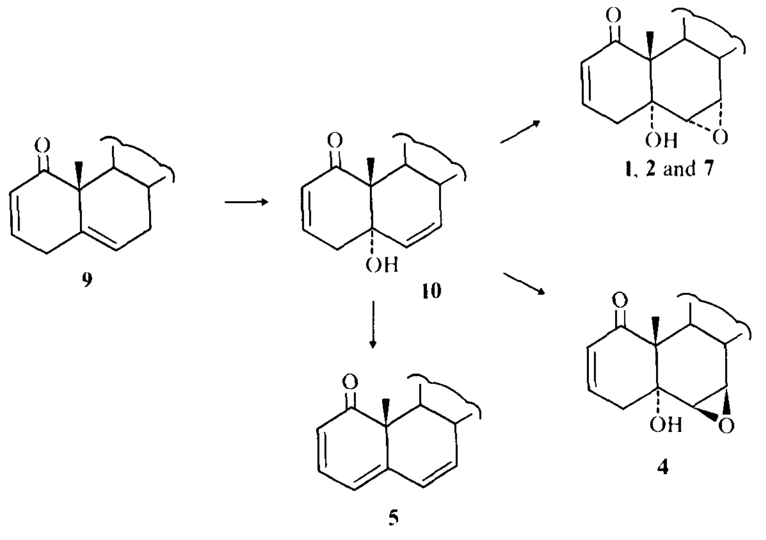
^{a,b,c} Values with the same superscript within any vertical column may be interchanged.

cross of chemotypes III or II with Indian I are now being analysed. Table 3 lists all the major compounds isolated from these two F_1 hybrids as a percentage of the total withanolide content. For comparison, the data of the relevant parents are also included. In Table 4 the combined quantitative amounts of each selected substituent in relation to the total withanolide content are presented, as percentages. Although each of them is treated in Table 4 as a separate item, other groupings are also obviously present at other sites of the molecule.

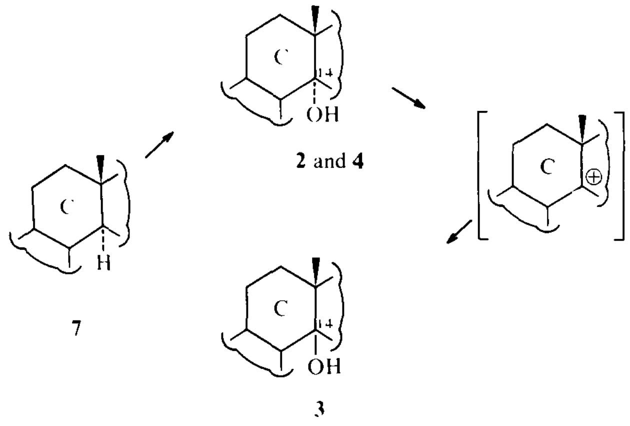
The relative yields of the 5α-hydroxyl and the 6,7α-epoxide are each *ca* 34 % in both hybrids. The remainder of the 66 % appears to be made up of two different components: one with the 4-desoxy-Δ^5-system and one consisting of a combination of three other components, viz. the 5,6β-epoxide, the 4β-hydroxyl (with Δ^5) and the 4β-hydroxyl (with 5,6β-epoxide). In the hybrid chemotype II (Israel) by Indian I, only the last substitution is present (64.4 %), while in that of chemotype III (Israel) by Indian I, the Δ^5-bond and the 5,6β-epoxide are present to the extent of 13.4 and 48.8 %, respectively. Considering the Δ^5 system as the probable precursor, one can see that in the hybrids deriving from chemotype II, which is known to have dominant characters, the disappearance of this precursor is very rapid, and therefore only 1 % of this system is recorded. Those deriving from chemotype III seem to have a slower reaction and, therefore, as much as 13.4 % of this precursor is observed. This is also to some extent true for chemotype III itself (8.1 %).

The same approach can also be extended to the substitutions in the rest of the molecule. Indeed, the computed values for 17α- and $20\alpha_F$-hydroxyl groups for both hybrids show the same relationship: 31.0 and 77.3 % against 27.2 and 82.0 %. The 17β-hydroxyl (α-side chain) is of very low occurrence and probably originates from a recessive gene. This is reflected by the fact that the 17β-hydroxylated structure is unique and not particularly widespread among the withanolides.

Considering the 14α-hydroxyl, the values of 1.0 and 12.5 % in the hybrids are in accord with those of the parent chemotypes, II and III. More important, a new gene may now be involved being responsible for the appearance of a 14β-hydroxyl (4.1 and 1.7 % in the two hybrids) which was not recognized so far in the two parents.



Scheme 1.



Scheme 2.

Table 3. Yields of withanolides given as a percentage of the total withanolide content in the parent and hybrid plants

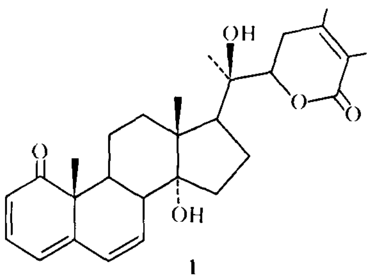
	Chemotypes				
Compound	Ind. I [5]*	II (Isr.) [13]*	III (Isr.) [14]*	II × Ind. I	III × Ind. I
Withanolide G			3.9	1.0	8.0
Withanolide I			0.4		2.4
Withanolide J			2.6		0.4
Withanolide U					2.4
Withanolide E			91.9		
Withaferin A	33.4				
Withanolide D		88.0		62.0	49.4
14-Hydroxy-20-desoxywithanolide D	0.9				
27-Hydroxywithanolide D		10.7		2.5	
Withanone	58.0			18.6	16.7
20-Hydroxy-17-desoxywithanone				3.6	6.3
Withanolide T				8.3	9.6
14α-Hydroxywithanone					0.3
14β-Hydroxywithanone				4.1	2.1
6,7-Desoxywithanone	0.9				

* The values are computed on the basis of the reported data.

Table 4. Calculated occurrence in percentage of each substitution in relation to the total withanolide content

	Chemotypes				
Substitution	Ind. I [5]*	II (Isr.) [13]*	III (Isr.) [14]*	II × Ind. I	III × Ind. I
Δ^5	1.1	0.3	8.1	1.0	13.4
4β-OH	35.5	99.6		64.4	51.2
5,6β-Epoxide	35.5	99.6	91.9	64.4	48.8
5α-OH	59.4			34.6	34.1
6,7α-Epoxide	58.5			34.6	34.1
14α-OH	0.9	1.0	99.4	1.0	12.5
14β-OH				4.1	1.7
17α-OH	63.8	0.3	3.4	31.0	27.2
17β-OH			92.3		0.8
$20\alpha_F$-OH		99.0	100.0	77.3	82.0

* The values are computed on the basis of the reported data.



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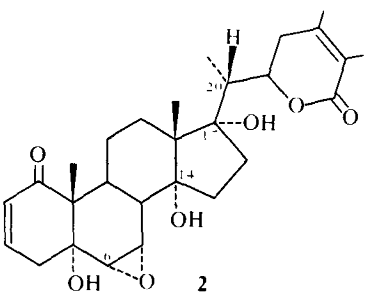

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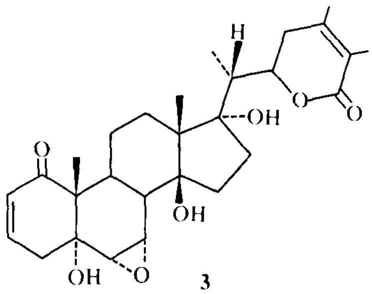

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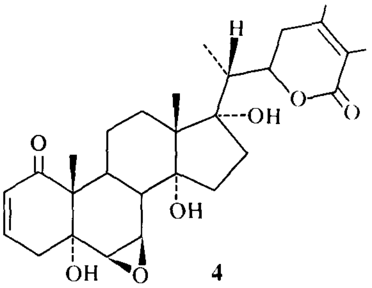

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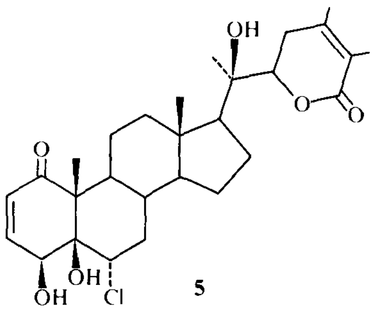

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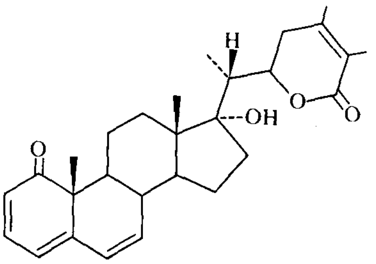

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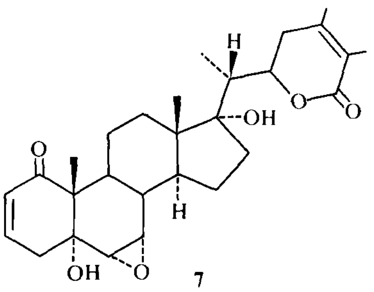

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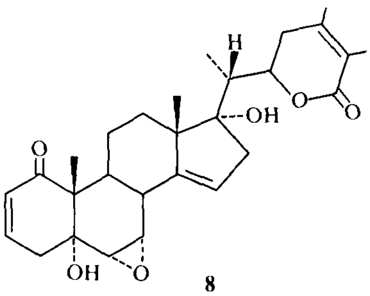

8

It is worth noting that during a study of the hybrids deriving from the cross of chemotype II with a type of *Withania somnifera* from South Africa [9], the dominant character known to be present in chemotype II, namely the 20-hydroxyl, prevailed. The double bond-reducing capacity, which was present only to a small extent in the South African plant [15], becomes major in the hybrids, which indicates that this capacity is actually a dominant character. Most of the withanolides of the offsprings contained a 20-hydroxyl and there was significant reduction of the double bonds either in the lactone ring or in ring A, the latter feature appearing as a new character.

EXPERIMENTAL

Mps were measured on a Fischer–Johns apparatus and are uncorr. IR spectra were recorded in KBr pellets; UV spectra were recorded for EtOH solns; ^{1}H NMR spectra were determined on a Bruker WH270 instrument (for 0.3–1.5% solns in $CDCl_3$ or in C_5D_5N where specified) and ^{13}C NMR spectra were obtained on a Bruker WH90 instrument, operating at 22.63 MHz for 2% solns in DMSO-d_6) with TMS as int. standard. For LC, a Si gel G60 (E. Merck) column was eluted with hexane–EtOAc mixtures and 1 l. fractions were collected. Analytical TLC was carried out on chromatoplates supplied by E. Merck

(50 × 75 mm, Si gel F_{254}). Mass spectra were determined under the direction of Dr. Z. Zaretskii. Analyses of the samples, dried under vacuum at 70° for 8 hr, were performed in the microanalytical laboratory of our Institute by Mr. R. Heller.

Isolation of withanolides from hybrids of Withania somnifera *chemotype* III (*Israel*) *by Indian* I (*Delhi*). The extraction of the plants was as reported earlier [2]. The isolation of withanolides from the various fractions by CC of the $CHCl_3$ extract is presented in Table 5. Some fractions contained more than one component but yielded the pure compounds by fractional crystallization.

Isolation of withanolides from Withania somnifera *chemotype* II (*Israel*) *by Indian* I (*Delhi*). The cross-pollination of the two parents was performed by Mr. A. Abraham. The leaves of the resulting F_1 hybrid plants (400 g) were used for extraction as before [2], using MeOH (3 l.), H_2O (1 l.) and $CHCl_3$ (2 l.). The extraction residue (10 g) was chromatographed on Si gel (500 g). The analysis of the fractions is shown in Table 6.

14α,20α$_F$-*Dihydroxy*-1-*oxo*-22R-*witha*-2,4,6,24-*tetraenolide* (**1**). Mp 220–222° from EtOAc; $[\alpha]_D$ +12.2° (*c* 0.3, $CHCl_3$); λ_{max} 352 and 228 nm (ε 3600 and 10 000); IR ν_{max}^{KBr} cm^{-1}: 3450, 1700, 1648, 1615, 1215, 1020 and 800. MS (HR) *m/z* (rel. int.): 452.2597 $[M]^+$ (14), 434.2449 $[M - H_2O]^+$ (21), 398.2247 $[M - 3H_2O]^+$ (3), 327.1978 $[M - C_7H_9O_2]^+$ (28), 309.1838 $[M - C_7H_9O_2 - H_2O]^+$ (24), 291.1751 $[M - C_7H_9O_2 - 2H_2O]^+$ (13), 251.1439 $[M - C_{10}H_{17}O_4]^+$ (55), 249.1273 $[M - C_{10}H_{19}O_4]^+$ (25), 171.0816 $[C_{12}H_{11}O]^+$ (100), 126.0670 $[C_7H_{10}O_2]^+$ (81), 125.0583 $[C_7H_9O_2]^+$ (52). (Found: C, 74.19; H, 8.19; $C_{28}H_{36}O_5$ requires C, 74.4; H, 8.03%. MW 452.3.)

5α,14α,17α-*Trihydroxy*-6α,7α-*epoxy*-1-*oxo*-22R-*witha*-2,24-*dienolide* (**2**). Mp 250° from $CHCl_3$–MeOH, 2:1; $[\alpha]_D$ +29.8° (*c* 0.3, $CHCl_3$–MeOH, 4:1); UV λ_{max}^{EtOH} nm: 226 (ε 11 800); ν_{max}^{KBr} cm^{-1}: 3480, 1698, 1680, 1140, 980 and 760; MS (HR) *m/z* (rel. int.): 486.2857 $[M]^+$ (1.3), 450.2455 $[M - 2H_2O]^+$ (1), 432.2286 $[M - 3H_2O]^+$ (4), 414.222 $[M - 4H_2O]^+$ (4), 343.1899 $[M - C_7H_9O_2 - H_2O]^+$ (18), 327.1972 $[M - C_7H_9O_2 - H_2O - O]^+$ (11), 325.1787 $[M - C_7H_9O_2 - 2H_2O]^+$ (5), 236.1195 $[C_{17}H_{16}O]^+$ (24), 223.1107 $[C_{16}H_{15}O]^+$ (23), 161.0981 $[C_{11}H_{13}O]^+$ (7), 125.0589 $[C_7H_9O_2]^+$ (100). (Found: C, 68.94; H, 7.92; $C_{28}H_{38}O_7$ requires C, 69.14; H, 7.82%; MW 486.3.)

5α,14β,17α-*Trihydroxy*-6α,7α-*epoxy*-1-*oxo*-22R-*witha*-2,24-*dienolide* (**3**). Mp 280° from EtOAc; $[\alpha]_D$ +36.7° (*c* 0.3, $CHCl_3$–MeOH 4:1); UV λ_{max}^{EtOH} nm: 226 (ε 11 400); IR ν_{max}^{KBr} cm^{-1}: 3540, 1695, 1681, 1400, 1150, 1000, 920, 810; MS (HR) *m/z* (rel. int.): 468.2478 $[M - H_2O]^+$ (1.5), 450.2398 $[M - 2H_2O]^+$ (8),

Table 5. Withanolides from chemotypes III (Israel) by Indian I (3.3 kg)

Fractions*	Eluant (hexane–EtOAc)	Withanolide present	Total yield (mg)
42–44	3:2	J	56
45–49	3:2	I	333
54–65	3:2	G	1106
68–69	1:1	2,4,6-Triene-1-one (**1**)	33
69–72	1:1	Chlorohydrin (**5**)	330
75–84	1:1	20-Hydroxy-17-desoxywithanone	858
88–105	1:1	D	6815
98–108	1:1	Withanone (**7**)	2210
112–125	1:1	T	1320
115–135	1:1	U	330
119–154	2:3	14α-Hydroxywithanone (**2**)	40
175–210	2:3	14β-Hydroxywithanone (**3**)	220
180–182	2:3	H	53
226–228	3:7	14α-Hydroxy-6,7β-epoxywithanone (**4**)	20
230–250	1:4	S	115

* The fractions were not combined; each was worked up separately to yield the indicated compounds.

Table 6. Withanolides from chemotypes II (Israel) by Indian I (400 g)

Fractions	Eluant (hexane–EtOAc)	Withanolide	Yield (mg)
8–10	3:7	G	5
13–17	2:3	20-Hydroxy-17-desoxywithanone	18
18–24	1:1	D	300
25–30	3:2	Withanone (**7**)	90
35–46	3:2	T	40
55–60	7:3	14β-Hydroxywithanone (**3**)	20
63–65	7:3	27-Hydroxywithanolide D	12

432.2259 $[M-3H_2O]^+$ (1), 325.1802 $[M-C_7H_9O_2-2H_2O]^+$ (100), 289.1593 $[M-C_7H_9O_2-4H_2O]^+$ (38), 125.0588 $[C_7H_9O_2]^+$ (36). (Found: C, 69.02; H, 7.90%. $C_{28}H_{38}O_7$ requires C, 69.14; H, 7.82%; MW 486.3.)

5α,14α,17α-*Trihydroxy*-6β,7β-*epoxy*-1-*oxo*-22R-*witha*-2,24-*dienolide* (**4**). Mp 242–244° from $CHCl_3$; $[\alpha]_D$ −41.8° (*c* 0.03, $CHCl_3$–MeOH, 1:1); UV λ_{max}^{EtOH} nm: 226 (ε 11 200) IR; ν_{max}^{KBr} cm^{-1}: 3400, 1698, 1685, 1370, 1200, 1050, 975 and 887; MS (LR) *m/z* (rel. int.): 468 $[M-H_2O]^+$ (4), 450 $[M-2H_2O]^+$ (25), 432 $[M-3H_2O]^+$ (8), 342 $[M-126-H_2O]^+$ (6), 337 $[M-149]^+$ (7), 327 $[M-125-H_2O-O]^+$ (13), 325 $[M-125-2H_2O]^+$ (100), 319 $[M-149-H_2O]^+$ (3), 307 $[M-125-3H_2O]^+$ (21), 289 $[M-15-4H_2O]^+$ (72), 125 (100). (Found: C, 68.86; H, 7.69. $C_{28}H_{38}O_7$ requires C, 69.14; H, 7.82%; MW 486.3.)

5α,17α-*Dihydroxy*-6α,7α-*epoxy*-1-*oxo*-22R-*witha*-2,14,24-*trienolide* (**8**). To compounds **2** or **3** (10 mg) in Me_2CO, conc. H_2SO_4 (2 drops) was added and, after 30 min, the soln was poured into H_2O (200 ml). The product was extrd with $CHCl_3$ (150 ml) and the residue, after crystallization from EtOAc, yielded **8** (8 mg); mp 278–279°; $[\alpha]_D$ +32.8° (*c* 0.12, $CHCl_3$); UV λ_{max}^{EtOH} nm: 227 (ε 10 880); IR ν_{max}^{KBr} cm^{-1}: 3450, 1705, 1690, 1450, 1377, 1188, 985, and 890; MS (LR) *m/z* (rel. int.): 468 $[M]^+$ (3), 450 $[M-H_2O]^+$ (8), 327 (6), 325 $[M-143]^+$ (100), 289 $[M-179]^+$ (35), 125 (9). (Found: C, 71.62; H, 7.92; $C_{28}H_{36}O_6$ requires C, 71.85; H, 7.75%; MW 468.2.)

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