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A Δ^{16}-WITHANOLIDE IN *WITHANIA SOMNIFERA* AS A POSSIBLE PRECURSOR FOR α-SIDE-CHAINS

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Key Word Index—*Withania somnifera*; Solanaceae; withanolides; steroidal lactones.

Abstract— The structure of a new naturally occurring steroidal lactone of the withanolide group isolated from *Withania somnifera* chemotype III has been elucidated as (20*R*, 22*R*)-14α,20α_F-dihydroxy-1-oxowitha-2,5,16,24-tetraenolid. This compound is considered to be an intermediate in the biosynthesis of withanolide E, and is at the origin of the unusual α-oriented side-chain in this compound. The comparative composition of withanolides in different sub-chemotypes of III is provided.

INTRODUCTION

Withanolide E (**1**), a steroidal lactone isolated from *Withania somnifera* chemotype III [1, 2], has been shown to exhibit antineoplastic activity and immunosuppressive properties [3]. For a better evaluation of its activity on a variety of experimental tumours, larger quantities were required. To this end a number of plant collections were made in the area of the institute and compared to samples of chemotype III grown in our experimental plots for the production of withanolide E. As a result, variations were observed in the concentration of **1**. In particular, one type, collected around the village of Yavne in the neighbourhood of our institute, showed a low concentration of **1** and seemed to be a different subchemotype. In this paper, we describe the analysis of these plants, and a comparison is made with those cultivated in our plots. The results of this study provide an insight into the origin of withanolide E (**1**) in the plant, especially with regard to the formation of its unusual α-oriented side-chain.

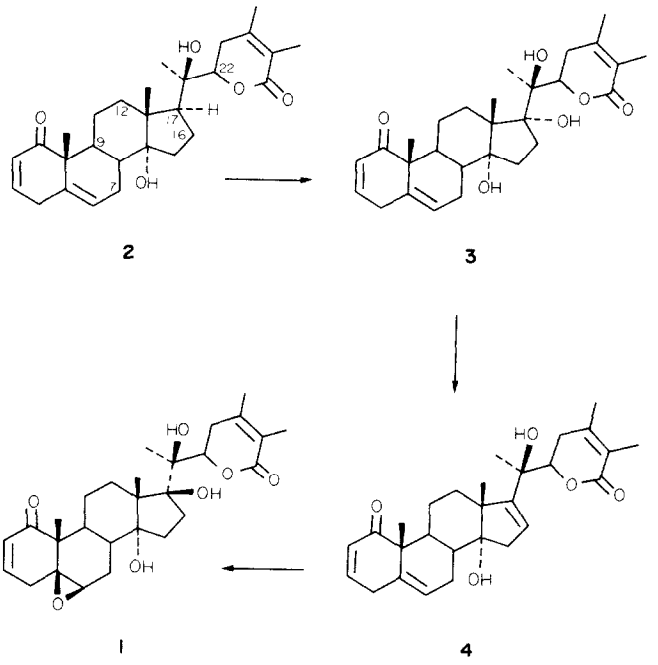
RESULTS

From the plants of chemotype III (Yavne), five compounds were isolated, namely withanolide E (**1**), and its corresponding 6,5-chlorohydrin [6, 7], withanolides G (**2**) and J (**3**) [4, 5] and the new Δ^{16}-withanolide (**4**) (Scheme 1). The quantities of these compounds in three different groups of chemotype III are given in Table 1.

The Δ^{16}-withanolide was assigned structure **4** on the basis of its ^{1}H and ^{13}C NMR data. On comparison of its ^{1}H NMR spectrum with that of other withanolides, three main differences were detected: a signal at δ 5.79 which was attributed to a vinylic proton on a trisubstituted double bond; the signal of H-22 which appeared at δ 4.45 (*dd*); and the signal of the 18-methyl group at δ 1.18. A double bond in ring D is found in withanolide L (**5**) [4, 5] and in the derived Δ^{14}-withanolide E (**6**) [2]. The values for these compounds are δ 5.27 and 5.23 respectively (**5** and **6**) (Scheme 2) and differ from the above value (5.79) probably because of the allylic-hydroxyl group. A similar system was found in holadysamine (**7**) [8] in which H-16 is at δ 5.66 and thereby supports the assignment of a 16, 17-double bond in the new compound. A ^{13}C NMR spectrum of a Δ^{16}-withanolide has not yet been reported [9] and the values given in Table 2 have been assigned by comparison with other withanolides, and by off-resonance ^{1}H decoupling. Evidence for the presence of a 14α-hydroxyl group in **4** was provided by the high field values observed for the signals of C-7, C-9 and C-12 (δ 25.5, 36.0, and 28.8), all undergoing a γ-effect induced by this 14α-hydroxyl group. The comparison can be made with the isomeric structure **6** where the values of δ 28.1, 43.4 and 36.0 were observed for these respective atoms. In compound **4**, the pentacyclic olefin resonates at a lower field (C-16 124.3; C-17 156.5) when compared with C-14 and C-15 of structures **5** (153.1 and 118.2), and **6** (152.4 and 114.3). All the other values for both ^{1}H and ^{13}C NMR spectra were characteristic for the steroidal structure of the withanolide group, and thereby confirm structure **4** for this compound.

DISCUSSION

The characteristics inherent to chemotype III seem to be the presence in all cases of a 20-hydroxyl group, the lack of a 4β-hydroxyl group, and the possibility of producing an α-side-chain. However, differences have been observed in the concentration of the components. Thus, as seen in three characteristic examples given in Table 1, the quantity of withanolide E may vary from 90 to 23% according to the subchemotype from which the leaves are collected. Column B of Table 1 provides the concentrations found in the cultivated plants grown from seedlings of



Scheme 1. Possible formation of withanolide E (1) from withanolide G (2).

Table 1. Comparative composition of withanolides in different sub-chemotypes of III

	Amount in % from total withanolides		
	A*	B*	C*
Withanolide G (2)	3.8	13	38.5
Withanolide J (3)	2.5	7	12.6
Δ^{16}-Withanolide (4)	—	—	2.1
Withanolide E (1)	90(0.525)†	77 (0.240)	23.0 (0.021)

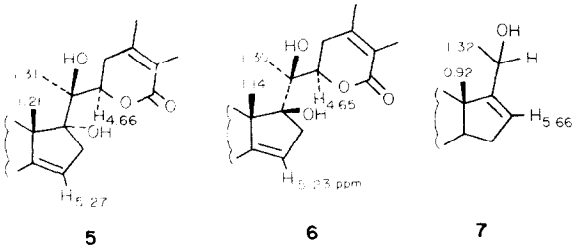
*A, best chemotype for production of 1 [4]; B, cultivated in experimental plots; C, Yavne type.

†Figures in parentheses give amounts as % from dry wt leaves.

Table 2. ^{13}C NMR data of compound 4 (22.63 MHz, $CDCl_3$, TMS as internal standard)

δ		δ		δ		δ	
C-1	198.0	C-8	34.5	C-15	40.0	C-22	79.5
C-2	128.2	C-9	36.0	C-16	124.3	C-23	29.9
C-3	145.2	C-10	50.7	C-17	156.5	C-24	149.2
C-4	33.5	C-11	22.3	C-18	22.5*	C-25	121.7
C-5	135.2	C-12	28.7	C-19	18.7	C-26	165.2
C-6	125.4	C-13	52.4	C-20	74.7	C-27	12.5
C-7	25.5	C-14	84.4	C-21	22.6*	C-28	20.6

*Interchangeable.



Scheme 2.

one of the types which turned out to produce an average of 77% of this compound in the total withanolide mixture. From the standpoint of withanolide E (**1**) production, the Yavne type is of no interest, but its constituents provided clues about the later biogenetic steps. The reduction in the formation of **1** went along with an increase of the other components. One of these was the Δ^{16}-withanolide **4** which is considered to be the direct precursor of withanolide E (**1**) and to be required for the inversion of the configuration of C-17. The sequence involving all the components present in this type is shown in Scheme 1: withanolide G (**2**) (38.5%) is converted to 17α-hydroxy-**2** (**3**) (12.6%) with retention of configuration, followed by elimination of the 17-hydroxyl group to form the Δ^{16} intermediate **4** (2.1%) which is then converted by hydration to **1** (23%) with inversion of configuration. In the other types producing higher quantities of withanolide E, the precursors occur in far smaller quantities and thus compound **4** was not detected until the present study. A similar sequence involving an inversion of configuration via a double bond has been postulated for the biosynthesis of digitoxigenin [10], and more recently for the formation of a 14β-hydroxyl group in a withanolide, which is unique in this group of compounds [11, 12].

EXPERIMENTAL

(20R,22R-14α,20α_F-*Dihydroxy*-1-*oxowitha*-2,5,16,24-*tetraenolide* **4**. Dry leaves (1.75 kg) of *W. somnifera* L. (Dun.) chemotype III, collected around the village of Yavne (mid-coastal plain of Israel), yielded, after the usual extraction procedure [4, 7], a residue (25 g) which was adsorbed on Kieselgel 60 (80 g) and chromatographed on the same material (600 g). 24 fractions of 500 ml each were collected. Fraction 11 (0.73 g) on chromatography yielded **4** (0.04 g), mp (uncorr.) 212–213° (from EtOAc), $[\alpha]_D + 44°$ ($CHCl_3$; *c* 0.34). UV λ_{max}^{EtOH} 225 nm (ϵ 23 700); IR ν_{max}^{KBr} cm^{-1}: 3560, 3460, 1710 and 1660; ^1H NMR ($CDCl_3$, TMS int. standard): δ 1.18 (3H, 18-Me), 1.24 (3H, 19-Me), 1.32 (3H, 21-Me), 1.88 and 1.97 (6H, 27 and 28-Me), 2.82 (1H, *dd*, $J = 20$, 4.5 Hz, H-4eq), 3.26 [1H, *d*(*br*), $J = 20$ Hz, H-4ax], 4.45 (1H, *dd*, $J = 11$, $J = 3.5$ Hz, H-22), 5.59 (1H, $W_{1/2} = 11$ Hz, H-6), 5.79 (1H, $W_{1/2} = 6.7$ Hz, H-16), 5.84 (1H, *dd*, $J = 9$, $J = 3.3$ Hz, H-2), 6.74 (1H, *dq*, $J = 9$, $J = 4.5$ Hz, H-3). MS *m/z* (rel. int.): 452 $[M]^+$ (0.3), 434 $[M - H_2O]^+$ (5), 416 $[M - 2 \times H_2O]^+$ (100), 309 [M − H_2O − 125 cleavage of C-20, C-22]$^+$ (50), 251 $[M - 2 \times H_2O - 125]^+$ (12), 125 (40). (Found: C, 74.12; H, 8.12%. $C_{28}H_{36}O_5$ requires C, 74.30; H, 8.02% MW 452.)

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