

## Withanolides Q and R, Two New 23-Hydroxy-steroidal Lactones

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Two new steroidal lactones of the withanolide type, isolated from *Withania somnifera* Dun (Solanaceae), are characterized by the unusual presence of a C-23 hydroxy-group. Withanolide Q (1a) is (22*S*,23*R*)-17 $\alpha$ ,23,27-trihydroxy-1-oxowitha-2,5,24-trienolide and withanolide R (2a) is (22*S*,23*R*)-6 $\alpha$ ,7 $\alpha$ -epoxy-5 $\alpha$ ,23-dihydroxy-1-oxowitha-2,24-dienolide.

In a previous paper<sup>1</sup> we dealt with the structures of eleven steroidal lactones of the withanolide series, isolated from the 'Indian I' chemotype of *Withania somnifera* Dun. The twelfth compound of this group, C<sub>28</sub>H<sub>36</sub>O<sub>6</sub>, isolated in minute quantities, was not at that time identified completely.

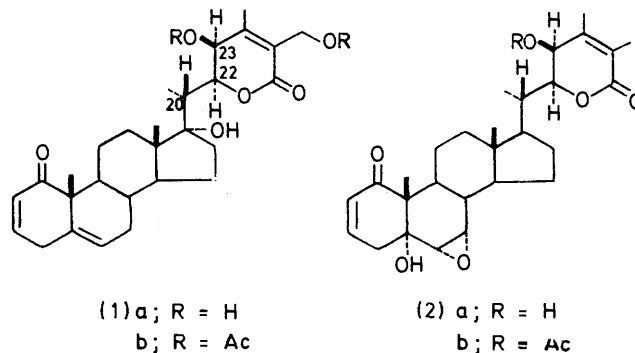
We have investigated recently the steroidal lactones produced in the offspring obtained by cross-pollination of the various chemotypes of *Withania somnifera*.<sup>2</sup> Crossing chemotypes I and III (both of which grow naturally in Israel) resulted in offspring F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub>, each containing an array of withanolides. Chromatography of the crude extract obtained from the leaves of one of the offspring F<sub>3</sub> afforded, *inter alia*, a new withanolide which proved to be structurally related to the twelfth compound of the Indian chemotype. The present work is concerned with the structures of these two compounds, designated withanolides Q and R.

Withanolide Q (1a) exhibits two i.r. bands in the carbonyl region at 1 650 ( $\alpha\beta$ -unsaturated ketone) and 1 705 cm<sup>-1</sup> ( $\alpha\beta$ -unsaturated  $\delta$ -lactone) and a u.v. maximum absorption at 218 nm ( $\epsilon$  17 900) attributable to the same unsaturated carbonyl chromophores. N.m.r. signals in the low-field region for three vinylic protons agree exactly with those displayed by withanolide G,<sup>3</sup> thus allowing the assignment of a 2,5-dien-1-one structure to the AB ring system. An unresolved band at  $\delta$  4.42 accounting for four protons and a singlet at  $\delta$  2.11 for a vinylic methyl group are assigned to the side chain.

<sup>1</sup> I. Kirson, E. Glotter, D. Lavie, and A. Abraham, *J. Chem. Soc. (C)*, 1971, 2032.

<sup>2</sup> D. Lavie, Nobel Symposium 25, Chemistry in Botanical Classification, Academic Press, New York and London, 1973, p. 181.

Acetylation of (1a) gave the diacetate (1b), in the n.m.r. spectrum of which the  $\delta$  4.42 band was completely resolved into a two-proton singlet at  $\delta$  4.95 (27-H<sub>2</sub>), a one-proton double doublet at  $\delta$  4.80 (22-H), and a one-proton doublet at  $\delta$  5.8 (23-H). The  $\delta$  4.95 singlet, in



conjunction with the single vinylic methyl signal ( $\delta$  2.11) defines the substituents at C-24 and C-25 as CH<sub>3</sub> and CH<sub>2</sub>·OAc. The double doublet at  $\delta$  4.80 points to two protons only in the neighbourhood of the 22-H; however, in contrast to the 22-H in the 20-hydroxywithanolides<sup>1,3</sup> the signal of which is split by the two C-23 protons with *J* 12 and 5 Hz, in the present case the coupling constants are only 3.5 and 2 Hz, excluding any axial-axial interaction. Deuteriation experiments on deoxydihydrowithaferin A<sup>4</sup> led to the exchange of the two C-23 protons and resulted in the appearance of the 22-H signal as a doublet (*J* 4 Hz). The only possible con-

<sup>3</sup> E. Glotter, I. Kirson, A. Abraham, and D. Lavie, *Tetrahedron*, 1973, **29**, 1353.

<sup>4</sup> D. Lavie, E. Glotter, and Y. Shvo, *J. Chem. Soc.*, 1965, 7517.

clusion is that in (1b) the axial proton at C-23 is replaced by an acetoxy-group, thus explaining also the doublet at  $\delta$  5.81 ( $J$  2 Hz) for the 23-H.

Double-resonance measurements confirmed the above interpretation: irradiation at  $\delta$  2.34 (20-H) leads to decoupling of the 21-H (singlet instead of doublet) and of the 22-H (doublet,  $J$  2 Hz, instead of double doublet); conversely irradiation at  $\delta$  4.80 (22-H) leads to decoupling of the 23-H (singlet instead of doublet) and of the

protons (21-H<sub>3</sub> and 22-H) which are near the 17-OH are shifted downfield. The Cotton effect in the c.d. spectrum of the lactone ring in (1a) is positive, as in all other withanolides (260.5 nm;  $\Delta\epsilon + 3.85$ ). Consequently (1a) is (22*S*,23*R*)-17 $\alpha$ ,23,27-trihydroxy-1-oxowitha-2,5,24-trienolide.

On the basis of the above data we can now assign a similar structure to the side chain of (2b) [the naturally occurring alcohol (2a) could not be isolated as such, but

N.m.r. signals \* of withanolides Q and R and their acetates

Compd.	2-H	3-H	6-H	7-H	22-H	23-H	19-H <sub>3</sub>	18-H <sub>3</sub>	21-H <sub>3</sub>	Other
(1a)	5.92dq (10, 3, 1)	6.86dq (10, 5, 2.5)	5.63 $W\frac{1}{2}$ 8							22-H, 23-H, 27-H <sub>3</sub> : broad band at 4.42; 28-H: 2.11s [2.31]
(1b)	5.90dq (10, 3, 1)	6.85dq (10, 5, 2.5)	5.63 $W\frac{1}{2}$ 8		[4.83] 4.80dd (2.5, 2) [5.21]	5.81d (2)	[1.22] 1.24s	[0.78] 0.79s	[1.26] 0.95d (7)	27-H <sub>3</sub> : 4.95s; 28-H <sub>3</sub> and acetates: 2.06, 2.09, 2.16
(2b)	5.85dq (10, 3, 1)	6.56dq (10, 4.5, 3)	3.05d (4)	3.33dd (4, 1)	4.73dd (3.5, 2)	5.63d (2)	[1.21] 1.17s	[0.78] 0.80s	[1.21] 0.93d (7)	27- and 28-H <sub>3</sub> : 1.97; AcO: 2.15

\*  $\delta$  Values:  $J$ /Hz in parentheses; data for solutions in C<sub>5</sub>D<sub>5</sub>N in square brackets.

21-Me; finally, irradiation at  $\delta$  5.81 (23-H) induces the decoupling of the 22-H (now doublet,  $J$  3.5 Hz).

Treatment of (1b) with basic alumina at room temperature caused elimination of the 23-acetoxy-group, to give the corresponding pyrone,  $\lambda_{\max}$  307 nm.

On the basis of the molecular weight of (1a), the compound must possess a third hydroxy-group which, in the absence of any relevant n.m.r. signal should be tertiary. It is assigned the 17 $\alpha$ -position by analogy with other withanolides and in agreement with pyridine-induced n.m.r. shifts  $\Delta$ (CDCl<sub>3</sub> - C<sub>5</sub>D<sub>5</sub>N) of 18-H<sub>3</sub> +0.01, 21-H<sub>3</sub> -0.26, and 22-H -0.41 p.p.m.<sup>1</sup>

Comparison of the n.m.r. spectra of (1a) and its diacetate (1b) (in CDCl<sub>3</sub>) reveals two relevant aspects concerning the protons near to the 23-substituent. In (1b), the 18-H<sub>3</sub> and the 21-H<sub>3</sub> signals are shifted upfield, whereas the 22-H signal is shifted downfield by 0.40 p.p.m. A model shows that the 23-acetate group restricts rotation about the C(20)-C(22) bond; as a result, in the most preferred conformation, the 18- and the 21-protons are more distant from the 23-substituent than in (1a), whereas the 22-H is held closer to the 17 $\alpha$ -OH, thus inducing deshielding of the latter and shielding of the former. The spectra of (1a) and (1b) in C<sub>5</sub>D<sub>5</sub>N support such an interpretation. Whereas in (1a)  $\Delta$ (CDCl<sub>3</sub> - C<sub>5</sub>D<sub>5</sub>N) is positive for 18-H<sub>3</sub> and negative for 21-H<sub>3</sub>, 22-H, and 28-H<sub>3</sub>, in (1b) 18-H<sub>3</sub> and 28-H<sub>3</sub> are practically unaffected, whereas the 21-H<sub>3</sub> and the 22-H signals are shifted downfield. It is known<sup>5</sup> that the effect of pyridine is due to hydrogen bonding with the hydroxylic oxygens. In (1a) the pyridine coordinated to the 23-OH restricts rotation about the C(20)-C(22) bond in such a way that the distance between the 23-OH and the 18-H<sub>3</sub> is increased (upfield shift of the 18-H<sub>3</sub> signal); concomitantly, the protons which are close to the 23-OH and the 17-OH are significantly deshielded. In (1b), where pyridine does not co-ordinate around the 23-acetoxy-group, only the signals of those

only as the 23-monoacetate]. Comparison of n.m.r. signals confirms that the only difference between the side chains in (1b) and (2b) is the absence of the 27-OH in the latter. As to the substitution pattern of rings A and B, and the similarity to (22*R*)-6 $\alpha$ ,7 $\alpha$ -epoxy-5 $\alpha$ ,17 $\alpha$ -dihydroxy-1-oxowitha-2,24-dienolide<sup>1</sup> leaves no doubt, about the structure (2b). Since the molecular weight of (2b) indicates the presence of only seven oxygen atoms, the compound possesses only one tertiary OH (5 $\alpha$ -OH).

The mass spectral fragmentation pattern of (1a) ( $M^+$  470) supports the assigned structure. The base peak ( $m/e$  267) is due to the cleavage of the C(17)-C(20) bond, accompanied by elimination of a molecule of water. Cleavage of the C(20)-C(22) bond, which is the dominant process in the fragmentation of withanolides unsubstituted at C-23, is less important in the present case and leads to the signal  $m/e$  295 ( $M^+ - 157 - 18$ ); the second possible fragment from this rupture, which would correspond to the lactone ring, is of low abundance. Nevertheless, there are two significant signals ( $m/e$  342 and 324) related to the cleavage of the lactone itself along the C(22)-C(23) and the O-C:O bonds.

In the spectrum of the diacetate (1b) ( $M^+$  554) the main signals correspond to the trivial elimination of water and acetic acid ( $m/e$  536, 494, 476, 434, and 416). Loss of the whole side chain (accompanied by elimination of water) leads to the same fragment,  $m/e$  267, as in (1a).

#### EXPERIMENTAL

M.p.s. were taken on a Fisher-Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in chloroform. I.r. spectra were recorded on a Perkin-Elmer Infracord 137 spectrophotometer equipped with a sodium chloride prism, and refer to KBr pellets; u.v. spectra were recorded on a Cary 14 instrument (ethanol as solvent); n.m.r. spectra were determined on a Varian A-60 spectrometer for 5-10% solutions in deuteriochloroform, containing tetramethylsilane as internal standard. T.l.c. was carried out on chromatoplates of silica gel G (Merck) and spots were developed with iodine vapour. Mass spectra were taken

<sup>5</sup> P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert, *J. Amer. Chem. Soc.*, 1968, **90**, 5840.

under the direction of Dr. Z. Zaretskii with an Atlas CH4 instrument. Analyses were performed in the micro-analytical laboratory of the Weizmann Institute, under the direction of Mr. R. Heller.

*Plant Material.*—Reciprocal cross-pollination of *Withania somnifera* Dun., chemotypes I and III, resulted in the offspring  $F_1$ . Self-pollination of  $F_1$  afforded several offspring  $F_2$  in which recombination of the chromosomes had taken place. One of these, which proved to be a new, artificial chemotype, remained unchanged when it was self-pollinated again. The offspring  $F_3$  thus obtained constituted the plant material of the present work.

*Isolation.*—Crushed air-dried leaves (2 kg) were exhaustively extracted with methanol and the extract was worked up as previously described.<sup>3</sup> The crude product (27 g) was chromatographed on a silica gel column (Woelm 0.063—0.1 mm; 3 kg). Elution with benzene-ethyl acetate (4.5 : 5.5) afforded crude withanolide Q (400 mg), which was purified on preparative chromatoplates (Woelm 60 F<sub>254</sub>; 2 mm thick). *Withanolide Q* (1a) (120 mg) (22S,23R)-17 $\alpha$ ,23,27-trihydroxy-1-oxowitha-2,5,24-trienolide had m.p. 200—202° (from ethyl acetate),  $[\alpha]_D -6.6^\circ$  ( $c$  0.12);  $\nu_{\max}$  1 650 and 1 705  $\text{cm}^{-1}$ ,  $\lambda_{\max}$  218 nm ( $\epsilon$  17 950); c.d. (ethanol;  $c$  0.63) 340 ( $\Delta\epsilon -3.14$ ) and 260.5 nm (+3.85) (Found: C, 71.45; H, 8.2%;  $M^+$ , 470.  $\text{C}_{28}\text{H}_{38}\text{O}_6$  requires C, 71.45; H, 8.15%;  $M$ , 470).

Acetylation of (1a) (50 mg) with acetic anhydride in pyridine, overnight at room temperature, gave the 23,27-diacetate (1b), m.p. 160—162° (from acetone),  $[\alpha]_D -88.8^\circ$  ( $c$  0.08);  $\nu_{\max}$  1 655, 1 715, and 1 745  $\text{cm}^{-1}$ ,  $\lambda_{\max}$  220 nm ( $\epsilon$  18 500) (Found: C, 69.3; H, 7.7%;  $M^+$ , 554.  $\text{C}_{32}\text{H}_{42}\text{O}_8$  requires C, 69.3; H, 7.65%;  $M$ , 554).

*Withanolide R Monoacetate* (2b).—Chromatography of the crude extract from leaves of *W. somnifera* 'Indian I' chemotype<sup>1</sup> resulted in the separation of several pure withanolides and in two inseparable mixtures designated A and B. Mixture A was acetylated with acetic anhydride-pyridine overnight at room temperature and the crude mixture of acetates (0.5 g) was separated by thick-layer chromatography on silica gel PF<sub>254</sub> (1 mm thick in benzene-ethyl acetate (2 : 8). Extraction of the third band from the top afforded (22S,23R)-23-acetoxy-6 $\alpha$ ,7 $\alpha$ -epoxy-5 $\alpha$ -hydroxy-1-oxowitha-2,24-dienolide (2b) (22 mg), m.p. 182—184° (from acetone-hexane),  $[\alpha]_D -64^\circ$  ( $c$  0.09),  $\nu_{\max}$  1 698, 1 712, and 1 745  $\text{cm}^{-1}$ ,  $\lambda_{\max}$  221.5 nm ( $\epsilon$  17 400) (Found: C, 70.6; H, 7.4%;  $M^+$ , 510.  $\text{C}_{30}\text{H}_{38}\text{O}_7$  requires C, 70.55; H, 7.5%;  $M$ , 510).

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