567. *Constituents of* Withania somnifera. *Part I. The Functional Groups of Withaferin.*

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Withania somnifera (Solanaceae) has yielded a new compound, withaferin $C_{21}H_{30}O_5$, containing two acylatable alcohol groups, of which one is primary, a ketone group, an αβ-unsaturated methyl ester grouping, a tetrasubstituted double bond, and a terminal methylene group.

Withania somnifera Dun. (Solanaceae) is a perennial herb native in India, South-Africa, and the Mediterranean region. In Israel it is abundant mostly in the southern part of the country which is hot and dry. In ancient medicine, it had a reputation of being narcotic and various healing properties were attributed to it.[1] Recently an antibacterial crystalline substance has been isolated from it.[2]

Power and Salway [3] have reported the isolation of a few substances from the leaves of *Withania* but did not characterise them; in an extract of the whole plant they were unable to detect any narcotic property. Majumdar [4] has examined the Bengal and South African varieties and claimed to have isolated six new alkaloids in addition to the substances previously reported, but no structural formulæ were reported for these substances.

The antibacterial substance recently isolated by Kurup [2] from this plant was given an empirical formula of $C_{24}H_{30}O_6$, and some physical constants were reported.

In our investigation we studied the constituents of the leaves of a variety of *Withania somnifera* growing in Israel. The crude methanolic extract of the crushed leaves was treated with aqueous acid and extracted with ether. The resulting nitrogen-free product was chromatographed on acid-washed alumina and five crystalline fractions were obtained. One of the major fractions was purified by repeated crystallisation, yielding a crystalline substance, m. p. 244—245°, $[\alpha]_D$ +17·9°, for which the name withaferin is proposed. This paper is concerned with its properties.

Analyses and molecular-weight determinations lead to an empirical formula $C_{21}H_{30}O_5$. Acetylation at room temperature afforded a diacetate indicating two acetylatable hydroxyl groups. The nature of a third oxygen atom was deduced from spectroscopic evidence. Among the bands in the infrared carbonyl region spectrum is one at 1694 cm.$^{-1}$, attributed to a ketone group, in conformity with a weak ultraviolet maximum at 285 mμ (ε 50). The rather low frequency of the infrared band can be related in this case to a hindered ketone. The ultraviolet spectrum showed also a maximum at 221 mμ (ε 6100), which, in conjunction with chemical evidence given below, indicates an αβ-unsaturated ester.[5] The position and intensity of the maximum, and chemical reactions described below, show the presence of a fully substituted endocyclic double bond in conjugation with a methyl ester group (for analogous systems see ref. 6). Hydrolysis of withaferin by base or acid gave methanol and a carboxylic acid (soluble in aqueous sodium hydrogen carbonate). Thus an infrared band at 1710 cm.$^{-1}$ in the spectrum of withaferin could be attributed to the αβ-unsaturated methyl ester grouping; in accordance with this, withaferin gave a positive hydroxamic acid test. Thus, the five oxygen atoms are accounted for.

In the nuclear magnetic resonance spectrum a sharp peak at τ 6·64 is related to the three protons of the methyl ester group; the signal at τ 9·30 is suggestive of an angular

[1] Chopra, Chopra, Handa, and Kapur, " Indigenous Drugs of India," 2nd edn., U. N. Dhur & Sons, Ltd., Calcutta, 1958, p. 436.
[2] Kurup, *Antibiotics and Chemotherapy*, 1958, **8**, 511.
[3] Power and Salway, *J.*, 1911, **27**, 53.
[4] Majumdar, *J. Indian Inst. Sci.*, 1933, **16***A*, 29; *Current Sci. (India)*, 1952, **21**, 46; *Indian J. Pharm.*, 1955, **17**, 158.
[5] Grewe and Bokranz, *Chem. Ber.*, 1955, **88**, 49.
[6] Nielsen, *J. Org. Chem.*, 1957, **22**, 1539.

methyl group, perhaps between five- and six-membered rings. A sharp peak at τ 8·7 probably signified another angular methyl group. A doublet at τ 9·11 (J 6·5 c./sec.) is attributed to a secondary methyl group ($>CH\cdot CH_3$), and one at τ 7·90 is believed to be due to an allylic methyl group ($>C{:}CR\cdot CH_3$) [7] in which the double bond is totally substituted, as indicated by the absence of any signals in the lower field which could be attributable to a vinylic proton (this supports the ultraviolet evidence). Two peaks, at τ 5·5 and 5·6, are attributed to a terminal methylene group; this group was also indicated by a band in the infrared spectrum at 896 cm.$^{-1}$. When withaferin in acetic acid was subjected to exhaustive ozonolysis the two double bonds were cleaved, and formaldehyde was formed; the large fission product was a methyl ketone, giving a positive iodoform test. It seems probable that the tetrasubstituted double bond has a methyl group as one of its substituents.

On oxidation with chromium trioxide in acetone, withaferin gave a monocarboxylic acid, but its diacetate was recovered unchanged, proving the presence of a primary alcohol in withaferin.

Experimental

Unless otherwise indicated, ultraviolet spectra were determined for ethanol solutions, infrared spectra for chloroform solutions with sodium chloride optics, and rotations for chloroform solutions at room temperature. M. p.s were determined on a Kofler block and are corrected. The nuclear magnetic resonance spectrum was determined at 60 Mc. in deuterochloroform solution with tetramethylsilane as internal reference; the line positions given are τ values.

Extraction of W. somnifera *Leaves.*—Air-dried leaves (1 kg.) were crushed and extracted (Soxhlet) during 8 days with methanol (5 l.); concentration to a small volume yielded a semi-solid, dark green residue. This was dissolved in acetic acid (300 ml.), and water was added, dropwise, to the solution, with stirring, until a total volume of 3 l. was obtained. The acidic aqueous suspension was then extracted with light petroleum (6 × 300 ml.) and became then a clear-reddish solution. The light petroleum extracts were rejected. The aqueous solution was extracted with ether (4 × 400 ml.), and the combined ether extracts were shaken several times with 10% aqueous ammonia and water. The ether extract was dried (Na_2SO_4) and evaporated, yielding a yellow, nitrogen-free (Dumas) solid (A) (4 g.).

The material (A) (8 g.) was placed in 99 : 1 chloroform–methanol on acid-washed alumina (Merck; 800 g.; 4 × 50 cm.). Elution with 99 : 1-chloroform–methanol (1 l.) gave an oil (0·5 g.). Elution with 98·5 : 1·5 chloroform–methanol gave the following fractions: 1 l., an oil (1·0 g.); 75 ml., A_4 (0·25 g.), m. p. 240° (from ethyl acetate); 75 ml., A_5 (0·25 g.), m. p. 271° (from ethyl acetate); 300 ml., A_1 (1·5 g.), m. p. 238—240° (from ethyl acetate); 100 ml., a mixture (0·3 g.); 500 ml., A_2 (2·5 g.), m. p. 243—245° (from ethyl acetate); 100 ml., a mixture (0·25 g.); 200 ml., A_3 (0·25 g.), m. p. 225—227° (from ethyl acetate). Finally, methanol (500 ml.) eluted amorphous material (1·0 g.).

Substance A_1, *Withaferin.*—Withaferin, crystallised several times from chloroform–ethyl acetate (1 : 3) (charcoal), formed needles, m. p. 244—245°, $[\alpha]_D$ +17·9° (c 0·52), $\lambda_{max.}$ 221 (ε 6100), 285 mμ (ε 50), $\nu_{max.}$ 1694 (C=O) and 1710 cm.$^{-1}$ (CaF_2 prism; in $CHBr_3$), and 896 cm.$^{-1}$ (KBr pellet), τ 5·5, 5·6, 6·64, 7·90, 8·70, doublet at 9·11 (J 6·5 c.p.s.), 9·30 (Found: C, 69·5; H, 8·35. $C_{21}H_{30}O_5$ requires C, 69·6; H, 8·3%).

Withaferin (200 mg.), left in anhydrous pyridine (3 ml.) and acetic anhydride (3 ml.) overnight at room temperature, gave a *diacetate* (200 mg.) that, crystallised from methanol, had m. p. 166—167°, $[\alpha]_D$ −12·1° (c 0·54), $\lambda_{max.}$ 210—220 mμ (ε 6000), $\nu_{max.}$ 1730, 1710, and 1240 cm.$^{-1}$ [Found: C, 67·15; H, 7·6; Ac, 12·8%; M (Rast), *ca.* 445. $C_{25}H_{34}O_7$ requires C, 67·2; H, 7·7; 2Ac, 13·2%; M, 447].

Basic Hydrolysis.—Withaferin (370 mg.) was heated in 4% aqueous sodium hydroxide (10 ml.) under reflux for 1·5 hr. It dissolved, and a red solution was formed. After cooling, about 2 ml. were distilled off, and 1 ml. of the distillate was used for a chromotropic acid test [8] for methanol that was positive. A similar test carried out according to Feigl [9] was also

[7] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 85.
[8] Boos, *Analyt. Chem.*, 1948, **20**, 964.
[9] Feigl, "Spot Tests, Organic Applications," Elsevier Publ. Co., Amsterdam, 1954, p. 244.

positive. The precipitate formed after acidification of the basic solution was soluble in 5% sodium hydrogen carbonate, thus indicating the presence of a carboxyl group.

Acid Hydrolysis.—Withaferin (830 mg.) was boiled in water (40 ml.) and concentrated sulphuric acid (1 ml.) for 2 hr., during which a liquid distilled over (about 20 ml.) and was collected. This distillate was saturated with sodium chloride and extracted with ether (3 × 15 ml.). The combined ethereal extracts were dried (Na_2SO_4), and from them a 3,5-dinitrobenzoate was prepared, m. p. and mixed m. p. with methyl 3,5-dinitrobenzoate 107·5°. The chromotropic acid test [8] performed on the distillate gave a positive reaction.

Chromic Acid Oxidation.—Withaferin (500 mg.), dissolved in acetone (distilled over potassium permanganate) (40 ml.), was treated at 0°, with stirring, with a mixture (0·8 ml.) prepared from chromic oxide (68 g.) in concentrated sulphuric acid (57 ml.) and water (100 ml.) and diluted with water to 250 ml.[10] The brown-red solution was kept at 0° for 3 hr. with continued stirring. After decomposition of the excess of the reagent with methanol, water (500 ml.) was added and the mixture extracted several times with chloroform. The combined extracts were washed with water and shaken several times with saturated aqueous sodium hydrogen carbonate. The combined aqueous carbonate extracts were acidified with 18% hydrochloric acid, whereupon a white precipitate was formed. This was collected, purified by repeating the above procedure, and dried over P_2O_5 for 24 hr. This gave a white microcrystalline *compound* (150 mg.), m. p. 145—146° (decomp.), $\lambda_{max.}$ 213—216 mμ (ε 6100) (Found: C, 64·6; H, 7·5. $C_{21}H_{36}O_6,H_2O$ requires C, 64·3; H, 7·2%).

Ozonolysis of Withaferin.—Withaferin (700 mg.) in glacial acetic acid (15 ml.) was treated with ozonised oxygen at 20° for 1·5 hr. Powdered zinc (1 g.) and water (10 ml.) were added and the mixture steam-distilled into a solution of dimedone (400 mg. in 10 ml. of 1 : 1 aqueous ethanol). After 24 hr. the precipitate was filtered off and recrystallised from dilute alcohol yielding formaldehyde dimethone (130 mg.), m. p. and mixed m. p. 189°.

A chromotropic acid test performed on the reaction mixture before steam-distillation was positive. The solution remaining after steam-distillation, containing the bulk of the molecule, was filtered, diluted with water (300 ml.), and extracted with chloroform. The extract was dried (Na_2SO_4) and evaporated, yielding a yellow solid (500 mg.) that gave a positive iodoform test.

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[10] Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548.