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A CHEMOTAXONOMIC STUDY OF *WITHANIA SOMNIFERA* (L.) DUN.*

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Abstract—Three chemical types of *Withania somnifera* (L.) Dun., Solanaceae, each containing different steroidal lactones of the withanolide type, have been found to occur in Israel; they have been called types I, II and III. Morphological differences could not be detected between the three types, although each of them has a definite and separate area of distribution. No qualitative ontogenetic changes in the withanolide content could be observed.

INTRODUCTION

POPULAR medicine, especially in India and South Africa,[1] has attributed many physiological properties to crude extracts of roots and leaves of *Withania somnifera* (L.) Dun. (Solanaceae). Sedative, hypnotic and antiseptic activity have been ascribed to these extracts, but Power and Salway,[2] the authors of the first scientific investigation on the constituents of this plant, could not confirm the presence of such physiological properties. Working on plant material collected in South Africa, they isolated from the roots, along with some nitrogen-containing components,† a compound $C_{25}H_{34}O_5$, described as withanol, and from the leaves, two other compounds, somnirol $C_{32}H_{46}O_7$ and somnitol $C_{33}H_{46}O_7$.

In 1956, Kurup[4] isolated from the leaves of *W. somnifera* collected in the Jamnagar region (India), a compound $C_{24}H_{30}O_6$, m.p. 157–159°, described as an "unsaturated lactone" possessing remarkable antibacterial properties, different however, from any of the substances described in the work of Power and Salway.[2]

A few years later, Dhalla, Sastry and Malhotra[5] failed to find Kurup's compound in the

* Contribution No. 1089-E 1966 series of the National and University Institute of Agriculture, Rehovoth.

† The alkaloidal constituents of *W. somnifera* have been investigated by many workers;[3] none of the isolated compounds possesses remarkable physiological properties.

[1] J. M. WATT and M. G. BREYER-BRANDWIJK, *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, p. 1010. E. S. Livingstone, Edinburgh-London (1962).

[2] F. B. POWER and A. H. SALWAY, *J. Chem. Soc.* **99**, 490 (1911).

[3] D. N. MAJUMDAR and P. C. GUHA, *J. Indian Inst. Sci.* **16A**, 29 (1933); D. N. MAJUMDAR, *Current Sci. (India)* **21**, 46 (1952); *Indian J. Pharm.* **17**, 158 (1955); A. ROTHER, C. K. ATAL, D. GOLD and A. E. SCHWARTING, *J. Chromatogr.* **5**, 178 (1961); K. L. KHANNA, A. E. SCHWARTING, A. ROTHER and J. M. BOBBITT, *Lloydia* **24**, 179 (1961); A. ROTHER, J. M. BOBBITT and A. E. SCHWARTING, *Chem. & Ind. (London)* 654 (1962); K. L. KHANNA, A. E. SCHWARTING and J. M. BOBBITT, *J. Pharm. Sci.* **51**, 1194 (1962); J. D. LEARY, K. L. KHANNA, A. E. SCHWARTING and J. M. BOBBITT, *Lloydia* **25**, 44 (1963); H. B. SCHRÖTER, D. NEUMANN, A. R. KATRITZKY and F. J. SWINBOURNE, *Tetrahedron* **22**, 2895 (1966).

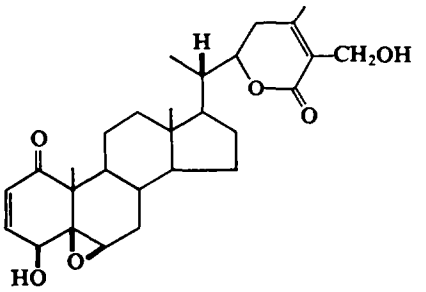
[4] P. A. KURUP, *Current Sci. (India)* **25**, 57 (1956); *Antibiot. Chemotherapy* **8**, 511 (1958); P. A. KURUP and J. MOHAN DAS, *Naturwissenschaften* **50**, 603 (1963).

[5] N. S. DHALLA, M. S. SASTRY and C. L. MALHOTRA, *J. Pharm. Sci.* **50**, 876 (1961).

leaves of *W. somnifera* collected also in India (in the Delhi region), however, they reported the isolation of somnitol and of a new compound designated as withanone $C_{24}H_{32}O_5$. Finally Kohlmuenzer and Krupinska[6] reinvestigated this plant and reported only the isolation of an unsaturated lactone which, according to them, is identical with the compound of Kurup.

Although none of the structures of all these compounds were elucidated, it is obvious that specimens of *W. somnifera* from various areas yielded different compounds.

Within the framework of a survey of the Israeli flora, the leaves of this species were thoroughly investigated for their content of the non-alkaloidal constituents. In the early stage of this work, the structure of the steroidal lactone withaferin A was elucidated.[7]* Since this compound could not be isolated from plant material collected in other regions of this country, a thorough study of this species was initiated in order to clarify the possibility of ontogenetic changes or the chemical variability of the species. Our interest in withaferin A and in related compounds is also due to their interesting physiological action. In addition to the remarkable bacteriostatic activity described first by Kurup[4] and confirmed by Ben-Efraim and Yarden,[8] this compound has also been found to have antitumour properties.[9]



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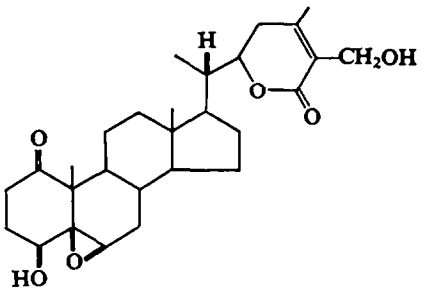

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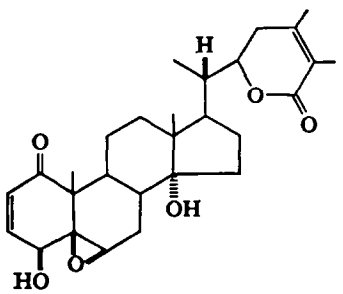

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* This compound, $C_{28}H_{38}O_6$, seems to be the same with the unsaturated lactone of Kurup; although withaferin A melts at 243–245° (from ethyl acetate), its melting point is 158–160° when crystallized from ethanol–water. The molecular weight (470) has been determined by mass spectrometry; the empirical formula, $C_{24}H_{30}O_6$, given by Kurup is due to wrong analytical results.

[6] S. KOHLMUENZER and J. KRUPINSKA, *Dissertationes Pharm.* **14**, 501 (1963); cf. *C.A.* **59**, 6866 (1963).

[7] (a) D. LAVIE, E. GLOTTER and Y. SHVO, *J. Org. Chem.* **30**, 1774 (1965); *J. Chem. Soc.* 7517 (1965); (b) E. GLOTTER, R. WAITMAN and D. LAVIE, *J. Chem. Soc.* (c) 1765 (1966).

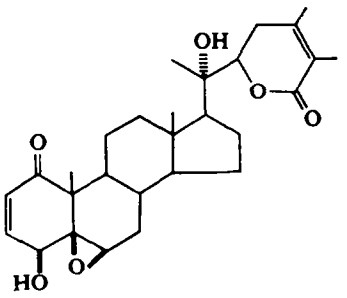
[8] S. BEN-EFRAIM and A. YARDEN, *Antibiot. Chemotherapy* **12**, 576 (1962).

[9] (a) S. M. KUPCHAN, R. W. DOSKOTCH, P. BOLLINGER, A. T. MCPHAIL, G. A. SIM and J. A. SAENZ RENAULD, *J. Am. Chem. Soc.* **87**, 5805 (1965); (b) B. SHOHAT, S. GITTER, A. ABRAHAM and D. LAVIE, *Cancer Chemotherapy Rep.* **51** No. 5, 271 (1967).

RESULTS

Leaves from samples of twenty four populations of *Withania somnifera* from various parts of Israel were examined for their content in steroidal lactones, and three well-defined chemotypes of this species were disclosed.

W. somnifera, chemotype I, contains predominantly withaferin A (1)[7a] (*ca.* 0·2 per cent of the oven dry leaves) accompanied by very small amounts of its 2,3-dihydroderivative (2) and of the 27-desoxy-14α-hydroxy isomer (3).[7b] Chemotype II contains a new compound (*ca.* 0·3 per cent) $C_{28}H_{38}O_6$, m.p. 253–255° $[\alpha]_D$ +80°* which has been characterized as (4).† In contrast with chemotypes I and II which have a limited number of steroidal lactones, chemotype III contains at least seven related components, some of which have already been characterized:



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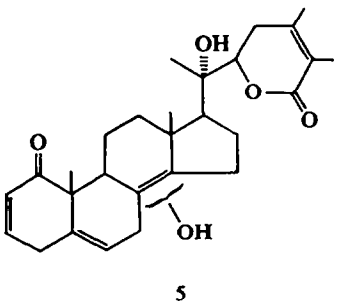

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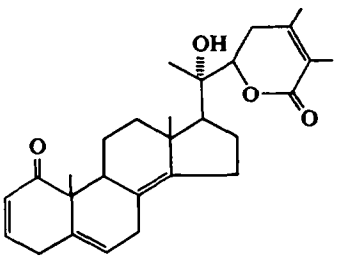

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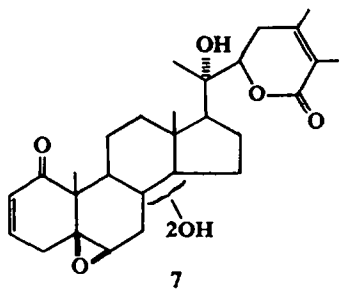

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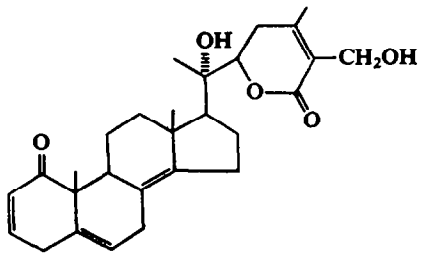

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(5) $C_{28}H_{36}O_5$, m.p. 186°, $[\alpha]_D$ +32·7° (*ca.* 0·05 per cent of dry leaves).

(6) $C_{28}H_{36}O_4$, m.p. 194–195° $[\alpha]_D$ +52·4° (*ca.* 0·05 per cent of dry leaves).

* Optical rotations are for chloroform solutions.

† The structure of this compound, which has been fully elucidated, will be demonstrated in a forthcoming publication.

(7) $C_{28}H_{38}O_7$ (*ca.* 0·2 per cent of dry leaves); this compound could not be induced to crystallize and was characterized as its dihydroderivative $C_{28}H_{40}O_7$, m.p. 273–275°, $[\alpha]_D$ −42·5°.

(8) $C_{28}H_{36}O_5$, also non-crystalline (*ca.* 0·01 per cent of dry leaves); it was characterized as its monoacetate, $C_{30}H_{38}O_6$, m.p. 141–142°, $[\alpha]_D$ +35·3°.

All these compounds belong to a new group of steroidal lactones for which the general name of *withanolide* has been proposed.[10] The individual components differ only in the substitution pattern; whereas the compounds isolated from the chemotypes I and II possess a secondary hydroxy-group at position 4, and a β-oriented epoxide at 5,6, all the compounds

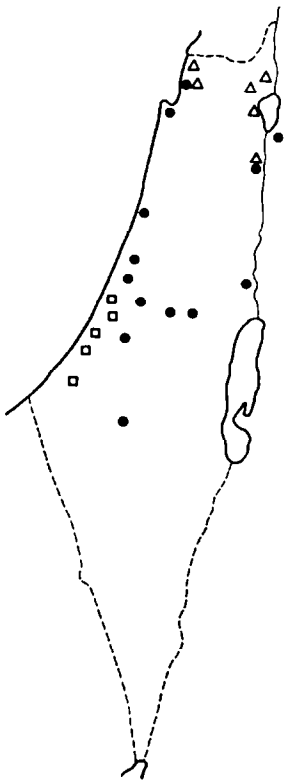

FIG. 1. GEOGRAPHICAL DISTRIBUTION OF THE CHEMOTYPES OF *Withania somnifera* IN ISRAEL.
● Chemotype I; △ chemotype II; □ chemotype III.

(5–8) isolated from chemotype III are not substituted at 4. The only compound in this group which possesses the 5,6-epoxy-ring is (7), while all other have a double bond at this position.

To clarify the possibility that chemotype III is not a mixture of single plants, each one containing various components, eight single plants taken at random from the area of distribution of this chemotype were examined. The results proved that each of these plants in this population contained all the components attributed to chemotype III.

The chemotypes of *W. somnifera* have a definite geographical distribution area. Chemo-

[10] D. LAVIE, S. GREENFIELD and E. GLOTTER, *J. Chem. Soc.* (C) 1753 (1966).

type II occurs in the northern part of Israel, chemotype III in the southern Coastal Plain, and chemotype I is distributed throughout the remaining parts of the country (see Fig. 1).

In order to examine the possible edaphic influence on the formation of withanolides in this plant, samples of each of the chemotypes taken from four different soil and moisture conditions were examined, but no significant differences could be detected. The constancy of withanolide content of the types was proven also in a direct way. Plants of all the types, raised from seeds in our uniform nursery, showed the same content of withanolides as those taken from their natural habitat.

In parallel with the above-mentioned work, the ontogenetic changes in the withanolide content were studied in plants of each of these chemotypes. For this purpose, plant material from each type grown in a uniform nursery was examined in five different phenological stages. It was concluded that no qualitative changes of the withanolide composition occurred during the growing period; there are, however, indications of some quantitative changes. In the stage of maximum vegetative development (occurrence of primordia of flowers) there seems to be a greater concentration of withanolides in the leaves.

A comparison of the morphological characteristics of the chemotypes was carried out on living plants of the same age which were grown in our nursery. After analysing a great number of characteristics, no difference could be found between the types. Only a difference in flowering time was observed, which may be of minor taxonomic value. In the nursery at Beit Dagan, chemotype III flowered 12 days earlier. This difference was observed on three subsequent sowing dates: 20 July 1966, 24 August 1966 and 5 April 1967.

DISCUSSION

Previous attempts to explain contradictory pharmacological results were based on the assumption that the species is variable; we could not however disclose any morphological variation in studying *Withania somnifera* of Israel origin. The same conclusion was drawn by studying herbarium specimens and different flora.

The only variation is of chemical nature, i.e. the content in withanolides of different substitution patterns. These studies conducted in a relatively limited area led to the identification of three distinct chemotypes; there are strong reasons to believe that additional chemotypes might be detected in the large area of distribution of this species* which might contain compounds such as withanone,[5] somnitol,[2,5] somnirol,[2] etc., reported by other authors. These compounds are not present in the chemotypes discussed in this paper. Although their structure has not yet been elucidated, it is possible that they belong also to the withanolide type of steroidal lactones.

It is noteworthy that withanolide type steroidal lactones have been recently isolated also from other genera of the Solanaceae family. Kupchan and co-workers[9a] have isolated withaferin A from *Acnistus arborescens*, a plant from Porto Rico, while Tschesche, Snatzke and co-workers[11] have obtained and characterized two related withanolides from *Jaborosa integrifolia* (from Paraguay) which were called jaborosalactones A and B. It is interesting to note that these two compounds do not possess the C_4-hydroxy group characteristic for the withanolides of chemotypes I and II.

* The distribution area of *W. somnifera* is from the Atlantic Ocean to South-East Asia and from the Mediterranean region to South Africa.

[11] R. TSCHESCHE, H. SCHWANG and G. LEGLER, *Tetrahedron* **22**, 1121 (1966); R. TSCHESCHE, H. SCHWANG, H. W. FEHLHABER and G. SNATZKE, *Tetrahedron* **22**, 1129 (1966).

The occurrence of such related compounds in different genera geographically so far apart, and the discovery of chemotypes in *W. somnifera*, require a thorough investigation of the steroidal lactones in the Solanaceae.

METHODS

The withanolide content of the plants was tested on whole populations consisting of fifty or more single plants. The sites of collection were chosen at random and at distances of at least 10 km from each other.

Extraction Procedure

Crushed oven-dried leaves of *Withania somnifera* collected in the areas shown in the map, or grown in the uniform nursery, were exhaustively extracted with methanol (soxhlet) (*ca.* 3 l. for 1 kg of plant material). The solution was concentrated to a volume of about 1 l. then 0·5 l. water was added and the mixture extracted with hexane to remove chlorophyll and other pigments. The residual methanol–water solution was then extracted with ether, the ethanol extract washed with water, dried (Na_2SO_4) and the solvent removed to leave a greenish foam-like residue (15 g). In the cases of plant material from chemotypes I and II, purification of the crude product was done by chromatography on acid-washed alumina (Merck). Elution with $CHCl_3$ (for chemotype I) yielded withaferin A (1) and dihydrowithaferin A (2) which were further purified by crystallization from ethyl acetate. From the mother liquors, compound (3) was obtained. Elution with $CHCl_3$–hexane (1:1) (for chemotype II) yielded the 20α-hydroxy isomer (4) which was then purified by repeated crystallization from ethyl acetate.

The crude product obtained from plant material belonging to chemotype III was chromatographed through silica gel H (Merck). The different compounds were eluted successively with benzene–ethyl acetate mixtures. At all these stages of purification, TLC on silica gel G (solvent mixture ethyl acetate–benzene, 7:3) was used for identification of the compounds. The spots were visualized with I_2 vapours.

In the cases of the chemotypes I and II the extraction procedure described by us in a previous publication[7] was also used, the results being similar to those obtained by the present procedure. Plant material from chemotype III could be processed only by this last procedure since part of the compounds, mainly (5), (6) and (8), are very sensitive.

Raising the Plant in Uniform Nursery

The seeds were rinsed with tap water for 2 days, then sown in our glasshouse (approximately 28°). Seedlings, 20–25 days old, of the three chemotypes were planted in our nursery (heavy alluvial soil, irrigated) evenly spaced, 60 × 60 cm, in three replications, forty plants per replica. For the examination of the ontogenetic changes of the withanolide content in the different chemotypes, plants grown in the nursery were harvested and analysed on five successive dates. The first harvest of leaves was done 70 days after germination, and subsequently once a month.

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