Im NMR-Spektrum der Substanz (100 MHz, $CDCl_3$) erkennt man zusätzlich zu den Signalen von drei tertiär gebundenen Methylgruppen ($\delta = 0{,}94$ (6H) und $\delta = 1{,}04$) und einem Olefinproton ($\delta = 5{,}71$) ein Multiplett bei $\delta = 3{,}67$ (2H), Breite 20 Hz, und ein fein aufgespaltenes Signal bei $\delta = 4.08$ (2H). Die Signale von zwei mit $D_2O$ austauschbaren Protonen um $\delta = 1{,}7$ sind kongruent mit der starken Hydroxylabsorption im IR-Spektrum. Somit lassen sich die beiden Multiplette im NMR-Spektrum als Signale von zwei Hydroxymethylgruppen interpretieren, die in den Teilstrukturen $-CH_2-CH_2OH$ bzw. $H-\overset{|}{C}=\overset{|}{C}-CH_2OH$ vorkommen. Acetylierung des neuen Metaboliten lieferte ein öliges Diacetat, $[\alpha]_D^{25} = +9°$, in dessen NMR-Spektrum die beiden Multiplette nach $\delta = 4{,}07$ bzw. $\delta = 4{,}51$ verschoben sind.

Das Vorliegen einer einfach ungesättigten carbobicyclischen Struktur mit 5 Endgruppen, worunter 3 tertiär gebundene Methylgruppen, liess eine biogenetische Beziehung zu Longifolen vermuten. Dies führte zwangsläufig zur Aufstellung von Formel **5**, deren Gültigkeit durch eine direkte Verknüpfung mit Longifolen erhärtet werden konnte. Behandlung des Diacetats **6** mit Natrium in Ammoniak lieferte den Monoalkohol **7**, $[\alpha]_D^{25} = +14°$, NMR: $H-\overset{|}{C}=\overset{|}{C}-CH_3$ $\delta = 1{,}54$. Als Nebenprodukt wurde ein Diol $C_{17}H_{30}O_2$ gefasst, Smp. 101°, $[\alpha]_D^{25} = -27°$, dessen spektroskopische Daten mit Struktur **8** kongruent sind. Die Entstehung von **8** lässt sich deuten mit der Annahme einer intramolekularen Alkylierung des intermediär gebildeten Allylanions durch die benachbarte O-Acetylgruppe, gefolgt von der Reduktion der C-Acetylgruppe[5]. Umsetzung von **7** mit Tosylchlorid in Pyridin lieferte eine rohes Tosylat, welches beim Behandeln mit Silicagel spontan cyclisierte. Das Produkt der Umsetzung, $[\alpha]_D^{25} = -46°$, wurde durch direkten Vergleich mit einer authentischen Probe als (−)-Longifolen, **4**, erkannt. Die Entstehung der neuartigen Secolongifolan-Verbindung **5** lässt sich durch die im Bild mit Pfeilen angezeigte Fragmentierung eines hypothetischen Vorläufers **9** deuten.

(−)-Longifolen ist bisher lediglich aus einem Lebermoos isoliert worden[6], während bei höheren Pflanzen immer wieder die antipodale (+)-Form angetroffen wird[7]. Mit (−)-Longifolen nahe verwandt ist das von *Fusarium culmorum* gebildete Culmorin, **10**[8]. Das in dieser Arbeit nachgewiesene Vorkommen von **2** und **4** in den beiden *Helminthosporium*-Arten erhärtet die phylogenetisch interessante Vorstellung, dass sich niedere und höhere Pflanzen unter anderem durch die Bildung von biogenetisch einheitlichen, zueinander antipodalen Reihen von Sesquiterpenen unterscheiden[9]. Das gleichzeitige Auftreten von (−)-Sativen, **2**, und (−)-Longifolen, **4**, in den beiden Ascomyceten als Gegenstück zum gleichzeitigen Vorkommen der entsprechenden Antipoden in Gymnospermen[10] ist unter diesem Gesichtspunkt besonders auffallend und weist auf die Möglichkeit hin, dass die Glieder eines gegebenen Paares jeweils aus einem gemeinsamen chiralen Vorläufer hervorgehen könnten.

*Summary.* (−)-Longifolene, **4**, and a new secolongifolane derivative, shown to possess structure **5**, have been isolated from *Helminthosporium sativum* and *H. victoriae*.

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## Variations in the Antitumour Constituents of *Withania somnifera* Dunal

*Withania somnifera* Dunal (Solanaceae) is a plant of repute (popularly known as Aswagandha) in the indigenous system of medicine in India. In Ayurvedic and Yunani system, the leaves of Aswagandha were used for tumours and tubercular glands[1]. Report[2] of the isolation of an antitumour steroidal lactone withaferin A (I) from the Israel variety of *W. somnifera* created a renewed interest in the plant. In course of further studies, it was revealed that specimens of *W. somnifera* from various areas yielded different compounds and 3 chemotypes of this species have been found to occur in Israel[3]. The chemotypes differ in the substitution pattern of the steroidal lactones of the withanolide type which have been isolated from the plant. Among these compounds withaferin A caused significant retardation of growth of Ehrlich ascites carcinoma, Sarcoma 180, Sarcoma Black, and E0771 mammary adenocarcinoma[2]. In view of the antitumour property and chemical variability of the plant, we were interested to make a thorough investigation of the Indian variety of *W. somnifera*. Recently, a study has been made with the plant occurring in the North-Western India and it is observed that *W. somnifera* of this region constitutes a different chemotype[4]. We report here our studies on this plant occurring in West Bengal and Tamil Nadu.

The leaves of *W. somnifera*, collected from West Bengal were defatted and extracted with methanol. The methanol extract on further fractionation involving repeated column chromatography and crystallization yielded a colourless crystalline compound (II), $C_{28}H_{38}O_6$, m.p.

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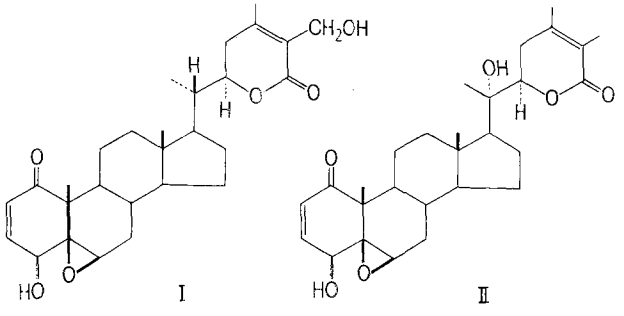
251–253°, $[\alpha]_D^{30°}$ + 80°($CHCl_3$). Yield, 0.03%. IR in $cm^{-1}$: 3500, 3390 (OH); 1709, 1680 (C = 0); *m/e* 470 ($M^+$). It formed an acetate, m.p. 245–248°. Preliminary studies showed that this compound was different from withaferin A, the antitumour lactone isolated previously. The compound has been identified as 4 *β*, 20*α*-dihydroxy-1-oxo-5*β*, 6*β*-epoxy-witha- 2,24-dienolide (II)[5] by comparison of physical constants i.e., mmp, specific rotation, thin layer chromatography (TLC) and finally by a superimposable IR-spectrum with that of an authentic sample. Compound (II) showed 95% tumour inhibition against Sarcoma 180 in mice[6] and was active against cells derived from human epidermoid carcinoma of the nasopharynx (KB), $ED_{50}$ 1.0 μg/ml (NSC No. 156284). No other steroidal lactones could be isolated from this source.




The petroleum ether extract of the leaves on column chromatography (Silica gel) yielded a crystalline material in the petroleum ether-benzene (1:1) eluate. The product, on repeated crystallizations from chloroform-methanol, gave colourless crystals, $C_{29}H_{50}O$, m.p. 138°–140°. IR in $cm^{-1}$: 3440 (OH). It responded to Liebermann-Burchard test for a sterol and formed a crystalline acetate, $C_{31}H_{52}O_2$, m.p. 127–129°, $[\alpha]_D^{30°}$ -41°($CHCl_3$). IR in $cm^{-1}$: 1740 (C=O) and 1250 (C–O, acetate); *m/e* 456($M^+$). The sterol was identical with *β*-sitosterol in all respects by mmp., TLC, and superimposable IR-spectra with that of an authentic sample.

The leaves of *W. somnifera*, collected from Tamil Nadu were examined. Following the same procedure as above, a colourless crystalline compound, $C_{28}H_{38}O_6$, m.p. 241–243°, $[\alpha]_D^{30°}$ + 114° ($CHCl_3$) has been isolated as the major product, yield, 0.04%. IR in $cm^{-1}$: 3350 (OH); 1709, 1685 (C=O); *m/e* 470 ($M^+$). It gave an acetate derivative, m.p. 201–202°. By comparison of the physical constants of the above product and its acetate with those of withaferin A and its acetyl derivative, the major product has been identified as withaferin A (I). All the above compounds gave satisfactory elemental analysis results. The TLC of the crude product after separation of withaferin A showed the presence of other minor constituents which could not be separated by column chromatography and crystallization. By preparative thin layer chromatography on silica Gel G in ethylacetate-benzene (7:3), the followign steroidal lactones could be isolated in trace amounts: 1. 27-Deoxy-14-hydroxy withaferin A; 2. dihydrowithaferin A; 3. 4*β*, 20*α*-dihydroxy-1-oxo-5*β*, 6*β*-epoxy- witha-2, 24-dienolide. The identity of these compounds was established by direct comparison of the authentic samples by mmp. and TLC. *β*-Sitosterol has also been found to occur in Tamil Nadu variety.

It may be mentioned that the plant occurring in Tamil Nadu contains predominently withaferin A (I) where as in the West Bengal variety compound (II) is the major antitumour constituent. The former specimen of *W. somnifera* conforms to chemotype I and the latter to chemotype II[3]. The plants studied were in the same developmental state.

*Zusammenfassung.* Nachweis, dass auch bei der indischen Varietät von *Withania somnifera* «chemische Rassen» mit unterschiedlicher Antitumoraktivität in verschiedenen Gegenden auftreten, die jeweils *β*-Sitosterol enthalten.

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## Hyaluronidase in the Mucus Accessory Glands of the Drone (*Apis mellifera*)

In a previous work[1], we reported on the properties of the hyaluronidase (hyaluronate glycanohydrolase E.C. 3.2.1.35) in crude extracts of the testes of the drone. In common with the mammalian testicular hyaluronidase, the insect enzyme was found to have the capacity of depolymerizing both hyaluronic acid and chondroitin-6-sulfate and to cleave these substrates at the hexosaminidic bond. The effects of pH were somewhat, and those of temperature definetly, different for the enzymes from the 2 sources. In initial experiments it came to our attention that extracts of the mucus accessory glands of the reproductive system of the drone also possessed hyaluronidase activity. The possibility of contamination with adjoining testicular tissue was, naturally, envisaged, but histological examination of several preparations of these glands and of their liquid content failed to reveal any such tissue or spermatozoa.

The presence of hyaluronidase in the insect accessory glands was considered of interest since such an activity has not been reported so far in the male accessory organs, and it might be connected with the special features of the fertilization process in the bee, as will be discussed later. The present paper reports on the hyaluronidase in

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