(—)-N-*Methyltetrahydropalmatine Iodide* (*Ib*).

(—)-*N*-Methyltetrahydropalmatine nitrate (130 mg) was dissolved in water (0·6 ml) and saturated KI solution (five drops) added. The resultant heavy white precipitate on recrystallisation from dry methanol yielded minute colourless needles (107 mg) m.p. 203–204°; $[\alpha]_D^{24}$ − 258·2° (C. 0·22 methanol); λ_{max}^{EtOH} 214, 232 (sh.), 284 nm (log ϵ 4·62, 4·34, 3·75) λ_{min}^{EtOH} 257 nm (log ϵ 2·98); Elemental analysis: Found: C, 53·13; H, 5·79. Calc. for $C_{22}H_{28}INO_4$: C, 53·10; H, 5·67%; when examined on cellulose chromatoplates (wet thickness 250μ) in 0·1 N HCl, a single spot (R_f 0·83) was obtained.

N-*Demethylation of* (—)-N-*Methyltetrahydropalmatine Iodide*

(—)-*N*-Methyltetrahydropalmatine iodide (20 mg) was vacuum distilled (260–280°/0·08 mm Hg) to yield a yellowish glass which on trituration with methanol yielded pale yellow needles (8 mg) m.p. 138–139°. The i.r. spectrum was identical with that of an authentic sample of tetrahydropalmatine (II) and gave identical R_fs on alumina and silica gel plates.

The u.v. spectra were recorded in ethanol and the i.r. spectra in KCl. PMR (60 MHz) spectra were recorded in the solvent indicated (Table 1) with T.M.S. as internal standard (τ10). Mass spectra were determined on an A.E.I. MS902 double-focussing mass spectrometer with the compound introduced *via* the direct insert probe. M.ps. (Kofler hot stage) are uncorrected. The optical rotations were measured in an 0·5 dm tube on a Pepol '60' spectropolarimeter. TLC plates were developed at 25°.

Acknowledgements—We thank Professor J. B. Stenlake, Dr. N. C. Dhar and Dr. R. D. Waigh for valuable discussions and for samples of norcoralydine, tetrahydropalmatine and tetrahydropalmatine methiodide. The mass spectra were recorded by Dr. P. Bladon, Department of Pure and Applied Chemistry, University of Strathclyde. The Forest Research Institute, Pretoria, Republic of South Africa is thanked for the supply and authentication of plant material.

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SOLANACEAE

5,20α(R)-DIHYDROXY-6α,7α-EPOXY-1-OXO-(5α) WITHA-2,24-DIENOLIDE, A NEW STEROIDAL LACTONE FROM *WITHANIA COAGULANS*

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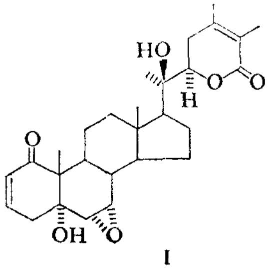
Abstract—The structure of the title compound (I) has been elucidated by analysis of the spectral data, as well as by its fragmentation pattern under electron impact. The configuration at C-20 (R) has been determined by NMR, and the one at C-22 (R) is based on CD measurements.

THE WITHANOLIDES are a series of C_{28} steroidal lactones with various substitution patterns, which have been isolated from different species of the Solanaceae family: *Withania som-*

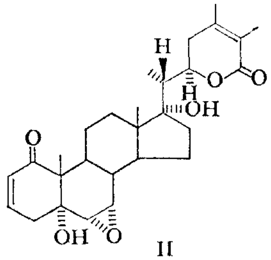
nifera,[1] *Acnistus arborescens,*[2] *Jaborosa integrifolia,*[3] *Acnistus australis*[4] and now, *Withania coagulans*. In this communication, we present the elucidation of the structure of the title compound (I) which has been isolated[5] from the roots of *Withania coagulans* together with withaferin A (V). It is noteworthy that this is the first instance that a withanolide is isolated from the roots of the plant; other members of this series described so far were obtained from the leaves. In the case of *W. somnifera* we definitely know that the roots do not contain steroidal lactones.

The characterization of I is based largely on comparison of its spectral data with that of an isomeric withanolide (II) earlier isolated in one of our laboratories,[6] the structure of which has been determined by unequivocal chemical degradation.

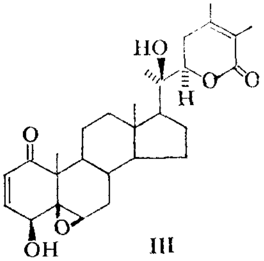
Compound I, $C_{28}H_{38}O_6$ shows two strong bands in the carbonyl region of the i.r. at 5·90 and 5·95 μ (KBr pellet), and an u.v. absorption at 223 nm (ϵ 14,000). Catalytic hydrogenation over $Pd/CaCO_3$ proceeds with the fast absorption of one mole of hydrogen yielding a 2,3-dihydroderivative, ν_{max}^{KBr} 5·85 and 5·95 μ and λ_{max} 227 nm (ϵ 7500). The low field NMR signals of the two vinylic protons in I (double quartets at δ 5·86 and 6·61 for the 2-H and 3-H, respectively) are not present in the spectrum of dihydro I. These data confirm the presence of two carbonyl chromophores, an easily reducible $\alpha\beta$-unsaturated ketone in ring A and an $\alpha\beta$-unsaturated lactone in the side chain. The two hydroxyl groups in I were



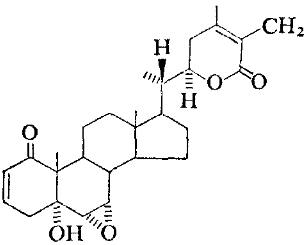
I



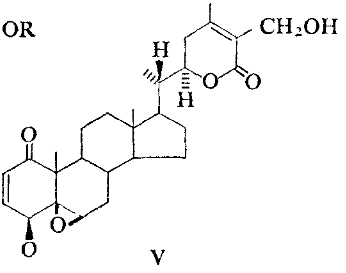
II



III



IV a. R=H
b. R=Ac



V

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(b) D. LAVIE, I. KIRSON and E. GLOTTER, unpublished results.

disclosed by the formation of a dicarbamate derivative with two moles of trichloroacetyl isocyanate (*in situ*,[7] in the NMR tube), characterized by a 2 × H signal at δ 8·55 for the N-H of the carbamates; the absence of any NMR signal which could be related to a proton adjacent to a hydroxyl function, and the failure of I to undergo acetylation under mild conditions, confirmed the assumption that the two hydroxyl groups are tertiary.

Comparison of the NMR spectra (100 MHz) of I and II ascertained their close relationship.

Compound		2-H	3-H	6-H	7-H	22-H	19-H	18-H	21-H	27 & 28-H
I	δ	5·86 dq	6·61 dq	3·05 d	3·33 dd	4·26 dd	1·18 s	0·95 s	1·31 s	1·93
	J	10; 3; 1 Hz	10; 4·5; 3 Hz	4 Hz	4; 1 Hz	12; 4 Hz				
II	δ	5·81 dq	6·60 dq	3·06 d	3·34 dd	4·63 dt	1·18 s	0·85 s	1·04 d	1·90
	J	10; 3; 1 Hz	10; 4·5; 3 Hz	4 Hz	4; 1 Hz	8·5; 3 Hz			7 Hz	

The differences between I and II are illustrated by the signals of the 22-H and the 21-methyl protons. The signal of 22-H in II is split by three neighbouring protons, giving rise to a double triplet pattern at δ 4·63; in I, the 22-H couples only with two neighbouring protons (at C-23), leading therefore to a double doublet. The appearance of the 21-methyl protons as a singlet confirms that no proton is linked at C-20; furthermore, the low field position of this signal (δ 1·31) suggests that one of the two tertiary OH groups is located at this carbon. Indeed, in the NMR of the bis-trichloroacetyl carbamate derivative, the 21-protons resonate at δ 1·95, i.e. a downfield shift of 0·61 ppm in comparison with the position of this methyl in I. Such a strong deshielding is possible only when the methyl and the esterified hydroxyl are on the same carbon. A similar shift for the 21-methyl (by 0·66 ppm) has been measured in the corresponding carbamate of III; the presence of a 20-OH substituent in III has been proven *inter alia* by chemical degradation to a pregnan-20-one derivative.

It is useful in this connection to note that in the bis-trichloroacetyl carbamate of II (17α-OH) the downfield shift of the 21-protons is only 0·22 ppm.[6b] The side chain of I has to be, therefore, similar to that present in withanolide D (III); this assumption is supported by the same trends in the solvent shifts ($\Delta^{CDCl_3}_{C_5H_5N}$) experienced by the 18, 21 and 22 protons in I and III.

		18-H	21-H	22-H
$\Delta^{CDCl_3}_{C_5H_5N}$ in ppm	I	−0·18	−0·10	−0·14
	II	−0·14	−0·11	−0·13

The location of the OH at C-20 is supported as well by the fragmentation pattern of I under electron impact, which is dominated by the cleavage of the bonds on the two sides of this hydroxyl. The peaks *m/e* 126, 125 and 345 (100, 46 and 17% respectively) are due to cleavage of the C_{20}-C_{22} bond. Cleavage of the C_{17}-C_{20} bond leads to the fragment *m/e* 169 (20 per cent relative intensity).

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The stereochemistry at C-20 (20α, Fieser's designation)[8] in III has been determined by comparing the effect of the 20-OH on the chemical shifts of the 18 and 21-protons, using an analogy with the 20-hydroxycholesterols.[9] The recent isolation[6b] from *W. somnifera* of a 20-desoxy derivative (IVa) enabled such calculations for I, as well.

$$\delta_{21\text{-H}}\,(\text{IVb}) - \delta_{21\text{-H}}\,(\text{I}) = -0{\cdot}26\text{ ppm;}$$

$$\delta_{18\text{-H}}\,(\text{IVb}) - \delta_{18\text{-H}}\,(\text{I}) = -0{\cdot}17\text{ ppm}$$

Similar effects are observed for 2,3-dihydro I and 2,3-dihydro-27-desoxy-IV, the corresponding effects being of −0·30 and −0·19 ppm respectively. These values compare favourably with those determined[10] for III and withaferin A (V) (−0·30 and −0·17 ppm for 21-H and 18-H respectively), as well as for cholesterol and 20α-hydroxycholesterol (−0·30 and −0·13 ppm respectively).[9]

Finally, the 22R stereochemistry [the same as in withaferin A (V)] has been determined by CD measurements.* Dihydro I shows a positive Cotton effect at 253 nm in the range of the R band of the conjugated lactone. The slightly positive Cotton effect at about 302 nm confirms the *trans* juncture of rings A/B. The α orientation of the C_5-OH is confirmed as well by the pyridine induced shift of the 19-methyl; since this group and the C_5-αOH are in a *trans diaxial* relationship, the $\Delta^{CDCl_3}_{C_5H_5N}$ should be very small and it is indeed only −0·02 ppm.

EXPERIMENTAL

M.ps were taken on a Fisher–Johns apparatus and are uncorrected Optical rotations were recorded with an automatic Perkin–Elmer 141 polarimeter and refer to $CHCl_3$ solutions. I.r. spectra were recorded as KBr pellets; u.v. spectra were recorded in EtOH solution; NMR spectra were determined on Varian A-60 and HA-100 spectrometers. Mass spectra were taken with an Atlas CH4 instrument.

Isolation Procedure

Dried, powdered roots of *Withania coagulans* Dun (collected in Northern India and authenticated by the Indian Botanical Survey, Calcutta), were extracted by percolation with methanol. The crude extract was concentrated in vacuum, water was added to the residue and re-extracted successively with petroleum ether and ether. The residue from the ether extract was chromatographed on neutral alumina (Merck). Elution with benzene yielded small amounts of I, further elution with benzene–$CHCl_3$ (1:1) and with $CHCl_3$ yielded withaferin A (V) (0·15% of the dried plant material) which has been identified by comparison with an authentic sample.

Compound I was further purified by TLC (chromatoplates of 1 mm thickness coated with silicagel PF_{254} Merck) in benzene–EtOAc (7:3) Crystallisation from EtOAc yielded pure I, m.p. 282–4°, $[\alpha]_D$ +88° (c 0·02), λ_{max} 223 nm (ε 14,000); ν_{max} 5·90 and 5·95 μ. Found: C, 71·55, H, 8·03, M^+ 470; $C_{28}H_{38}O_6$ requires: C, 71·46; H, 8·14%; m.wt. 470·58).

Attempted acetylation with Ac_2O and pyridine, over night at room temperature, left the compound unchanged.

Hydrogenation of I

Compound I (20 mg) in ethanol solution (10 ml) was hydrogenated over 10% $Pd/CaCO_3$ (5 mg) during 0·5 hr at room temperature and atmospheric pressure. The product was crystallised from EtOAc, m.p. 290–91°, $[\alpha]_D$ +128·5° (c 0 03); λ_{max} 227 nm (ε 7500); ν_{max} 5·85 and 5·95 μ. NMR δ 0·95, 1·20 and 1·31, singlets for 18-H, 19-H and 21-H respectively, 1·91 signal for 27 and 28 vinylic methyl groups, 3·05 (d, $J = 4$ Hz) for 6-H, 3·28 (dd., $J = 4$, 1 Hz) for 7-H, 4·21 (dd, $J = 12$; 5 Hz) for 22-H. CD (ethanol) λ_{max} (Δε): ~302 inflection (+0 35), 253 (+4 32), 198 (+16). Found· M^+ 472; $C_{28}H_{40}O_6$ requires· m.wt. 472·60.

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* We are grateful to Dr. G. Snatzke, Bonn, for these measurements.

[8] L. F. Fieser and M. Fieser, *Steroids*, p. 344, Reinhold, New York (1959).

[9] A. Mijares, D I. Cargill, J. A. Glasel and S. Lieberman, *J. Org. Chem.* **32**, 810 (1967)

[10] D. Lavie, I. Kirson and E. Glotter, *Israel J. Chem.* **6**, 671 (1968); keeping Fieser's designation,[8] the stereochemistry at C-20 should be drawn as shown here (compound III).