5. DISCUSSION
Fruits of *Withania coagulans* Dunal have been reported to be useful in a number of ailments and to confirm these facts, some pharmacological studies have been reported with the crude extracts of the fruits. Some attempts have also been made to isolate the active principles in pure form. But except for pharmacologically inert substances like proteolytic enzymes, triacontane and dihydrostigmasterol, (and some alkaloids in crude form), no chemical constituent had been isolated from the fruits in pure form to which the actions reported with the crude extracts and the medicinal uses of the fruits could be ascribed. The possible reason could be that in addition to the active principles like alkaloids and steroidal constituents, the fruits are rich in proteins, amino acids, oil, colouring matter, sugars and inorganic constituents which hinder the isolation of active principles in pure form.

In view of the biological activity of the crude extracts of the fruits and the recent reports about the isolation of biologically active steroidal lactones called withanolides from the roots and leaves of this
An attempt has been made to isolate similar type of chemical constituents and any other constituent from the fruits of *Withania coagulans* also.

Preliminary investigations indicated that petroleum ether, alcohol and water extracted substantial amounts of the constituents. Petroleum ether extract was oily in nature, aqueous extract was rich in proteins, sugars, alkaloids, steroidal constituents and colouring matter whereas alcoholic extract contained all the constituents except proteins. All the three extracts were therefore, investigated for the isolation of the chemical constituents. It was further found that like *Withania somnifera*, the fruits of *Withania coagulans* were rich in alkaloids and steroidal constituents. As many as 7 steroidal compounds, one triterpene, 2 hydrocarbons, one long chain fatty alcohol and 3 alkaloids have been isolated in crystalline form, though many more were detected in crude extracts. In addition, other constituents like sugars, fatty acids and inorganic ions have been identified in respective extracts.

By the use of paper chromatography, chemical tests, preparations of derivatives and quantitative
estimation, the fruit pulp was found to contain 3.5% sucrose, 4.48% glucose and glucuronic acid. Salam and Wahid (1969) using paper chromatography, detected D-arabino-
nose, D-galactose, maltose and galactoaraban in the seed. However, the presence of these sugars could not be confirmed in the present study as the purified aqueous extract of the fruit pulp and seeds failed to give Tauber reaction, a specific colour reaction for pentoses and mucic acid test specific for galactose (Jacob, 1962 b). By specific chemical tests and paper chromatography; the presence of sucrose, glucose and glucuronic acid was confirmed in the fruit pulp and seeds. The cause of this discrepancy could not be explained by the present study. The only possible explanation for this discrepancy seems to be that like Withania somnifera as reported by Abraham et al (1968), Withania Coagulans is also of many chemo types.

From the roots and leaves of W. coagulans and W. somnifera, a number of withanolides have been isolated from the methanol extract of these drugs. Methanol extract of Withania coagulans fruits also gave strong positive test for steroidal constituents but these being rich in oil, sugars
and colouring matter, no withanolide could be isolated from this extract by the procedure described by Kirson et al (1971), Subramanian and Sethi (1969). Taking the idea from the facts that the steroidal constituents are excreted in urine, especially during pregnancy (Zondek, 1928; Butendieck, 1929; Doisy et al, 1929, 1930; Murad and Gilman, 1975); the steroidal constituents were extracted from fruits with water. Chloroform extract of the water macerate gave strong positive tests for steroids. Two new withanolides and one steroidal glucoside have been isolated from the fruits of *Withania coagulans* by this procedure.

The seed and husk oils have different physical constants and chemical composition. A volatile oil is present in husk oil, whereas the seed oil is rich in unsaturated fatty acids. In view of the high content of linoleic acid, low amount of saturated acids and high iodine value, seed oil can be classified as an oil containing essential fatty acids which prevent atherosclerosis and high cholesterol accumulation (Mead et al, 1958; Bamberidge et al, 1958). As the proteolytic enzyme is being explored for cheese manufacture, the seed oil obtained as byproduct may find use for this purpose. The composition
of the main fatty acids of the seed oil found by spectroscopic studies by us is in agreement with the fatty acid composition found by Salam et al (1969) by GLC of methyl esters of mixed fatty acids.

From the unsaponifiable matter of the fruits oil, 4 steroidal compounds, one triterpene, one saturated hydrocarbon and a long chain fatty alcohol have been isolated.

Structural elucidation of the steroidal constituents have revealed that a number of them are new and have not been reported from any plant source so far. The withanolide, m.p. 260-261°, identified as 1-oxo-3β, 14α, 17β, 20αF-tetrahydroxy-20S, 22R-wita-5,24-dienolide or 3β-hydroxy-2,3-dihydro-withanolide F, has an α-oriented side chain which is so far unique in nature for steroidal structures. Second withanolide, m.p. 190-192° has been identified as 1-oxo-3β, 14α, 20αF, 27-tetrahydroxy-20R, 22-R-wita-5,24-dienolide or 3β-hydroxy-2,3-dihydro-withanolide H. Both these withanolides are new and of high biogenetic interest. The sterol, m.p. 208-209° identified as 3β, 24δ-dihydroxy-ergosta-5,25-dienolide is
also new and is a very early precursor in the biogenetic pathway of withanolide skeleton.

In a series of papers published (Kirson et al., 1977; Eastwood et al., 1980; Mittala and Lavie, 1981) dealing with the study of withanolides in different hybrids obtained by selected cross-breeding, in addition to genetic studies at the chemical level, it was possible to account for biogenetic pathways based on the structure of the different components fitting into the proposed sequence (Vande Velde and Lavie, 1981; Vande Velde and Lavie, 1982). Studies on additional chemotype or species, may contribute and support the ideas presented at different occasions. For example, it has been previously suggested that ring A of the withanolide is produced following a sequence a-d, shown in scheme 2. Presently, the study of W. coagulans and the isolation of 3β-hydroxy-2,3-dihydro-withanolide F from natural sources having the substitution shown in C and its conversion to J, provided now the complete pattern and thereby all the combinations shown in a to d have now been actually isolated from different withanolide producing plants.
Similarly presence of a 1-keto-3-hydroxy system in the compound IIIa is the second case for the occurrence of such a system in our studies on \textit{W. coagulans}. To the best of our knowledge, only once before such a system was referred in a plant (Vande Velde and Lavie, 1981). Now a precursor for the 2-en-1-one system of the withanolide is being fully described both for the compounds having the side chain \( \alpha \)-oriented (compound Ia) and the second for a regular \( \beta \)-oriented side chain(IIIa).

Presence of 1-keto-3-hydroxy system, follows the biogenetic sequence described in scheme 2. Furthermore, it is our present belief that elimination of 3-OH(as \( \text{H}_2\text{O} \)) can take place along two lines: one by elimination towards 0-2 to produce the 2-en-1-one system forming withanolide H(IVa) or secondly, towards 0-4 to produce the 3-en-1-one system(conjugated to \( \Delta 5 \)) producing
Scheme 3
the \( \Delta^3 \)-isomer of withanolide H (compound 2 of Vandae Velde and Lavie, 1981). Presence of the latter system was also found in other withanolides isolated from \textit{W. somnifera} (Glotter et al., 1973; Kirson and Gottlieb, 1980). Further the compound \( A_3 \) has been found to be an interesting case of containing primary, secondary and tertiary alcohol functions.

Along the same lines, the identification of sterol \( W \) is of importance and may provide additional information and the clue to the way whereby the lactone side chain is formed in the plants. 24-Methylenecholesterol, \( g \) in the scheme 3 has been shown to be a precursor and at the origin of the \( \delta \)-lactone side chain of the withanolide (Lockley et al., 1974). It is now proposed that compound VI (\( b \) in the scheme 3) is actually one of the intermediate steps leading to the lactone. In such a case, \( b \) should undergo 22-hydroxylation to \( g \), a well known reaction in the steroidal pathway in nature (Glotter et al., 1978 b). From this point two alternative hypotheses may be considered as shown in the scheme 3, one going through cyclization (\( d \)) and oxidation (\( e \)); and the second through oxidation (\( g \)) of
the allylic isomer(f), followed by cyclization to (g).
The latter compound, a lactol, has been found to be present
in a number of withanolides (Begley et al., 1972; Bates and
Morehead, 1974; Begley et al., 1973; Kirson et al., 1980).
Ultimate oxidation lead to the α-lactone(h).

Compound \( A_4 \) has been identified as \( \beta \)-sitosterol-
\( \beta \)-D-glucoside. Though it is not a new compound but is of
rare occurrence in plant kingdom. It is surprising to record
that though roots and leaves of \( W. coagulans \) have been found
to contain withaferin A, none of the 7 steroidal compounds
isolated from fruits correspond to withaferin A. It can
be presumed that either it is absent from fruits or is
present only in traces.

So far the alkaloids are concerned as many as
13 alkaloidal components have been detected in fruits, 7
in seeds and 10 in leaves by TLC studies. Of these, two
alkaloids m.p. 232-233° (already reported by Gaind and
Budhiraja, 1967) and m.p. 255° have been isolated in
small quantities as crystalline bases. Another water
soluble alkaloid has been isolated as picrate; m.p.
270-271°; remicratate, m.p. above 350° and picrocolonate, m.p. above 288°. Other alkaloids could not be purified even by conversion into salts due to their weak basic nature.