

Extraction of hecogenin from sisal (*Agave Vera-cruz*) leaves

M. S. MURTHY, A. K. VAISH AND S. RAJAGOPALAN

Department of Chemical Engineering, Indian Institute of Science, Bangalore 560 012, India.

Received on April 3, 1981.

Abstract

Preliminary investigations on the utilization of sisal waste give a hecogenin yield of about 0.1% of the leaf weight, with the maximum amount being present in the sediment of the juice.

Key words: Hecogenin, extraction.

1. Introduction

Sisal plant (Fig. 1) is grown mainly in Africa and Brazil. *Agave Vera-cruz* is one of the seventy-five varieties of general *Agave* family. In India, it is found in Assam, Bihar, Bengal, Madras, Maharashtra, Andhra Pradesh and Karnataka, *Agave Vera-cruz* is grown in abundance in waste lands and landscapes particularly in dry climates of Andhra Pradesh, Karnataka and Maharashtra.

Agave was introduced to India around 1830 by Portuguese and was used mainly for hedges along the railway track, paddy and other fields. Until recently, it has mainly been a source of fibre, the yield of which is around 3-4% of the weight of the leaves. Remaining 96% is left unutilized and thus wasted.

Sisal leaves are light green in colour with a prominent ashy shade. The leaves grow from a central bud to about 70 to 150 cm long, 15-25 cm wide and 0.5 cm thick at the centre. The leaves have thorns on the edges with sharp tips. In addition to fibre of high strength, sisal leaves are a potential source of more useful products such as hecogenin and wax.

Hecogenin is used as a base chemical in the manufacture of steroid drugs and hormones. In 1979, the hecogenin demand in India was roughly estimated at 20 tons. At present

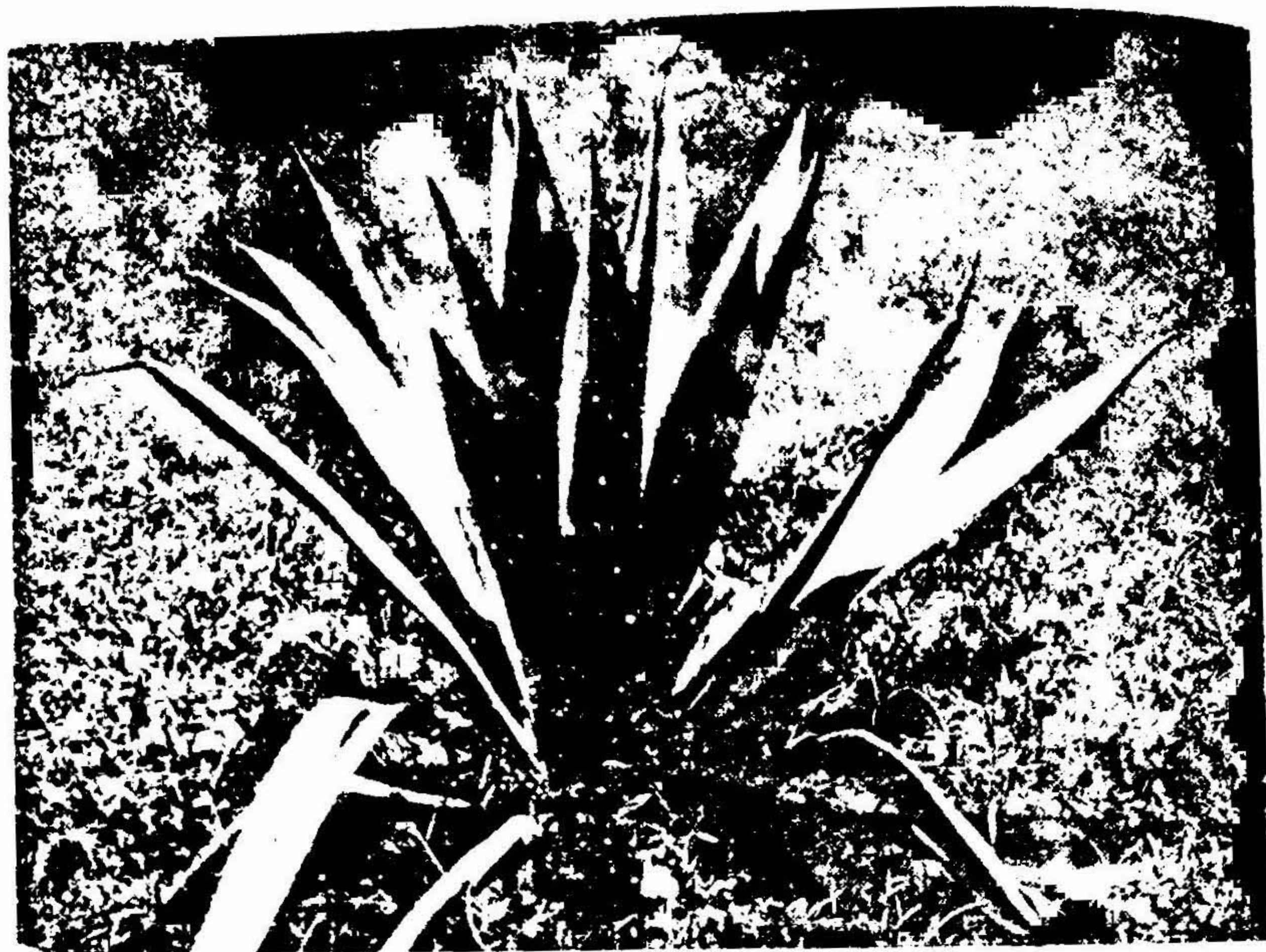


FIG. 1. Sisal plant.

it is being completely imported. Hecogenin, $C_{27}H_{42}O_4$, a steroidal sapogenin is present in the leaves of many *Agave* plants along with tigogenin. The ratio of hecogenin to tigogenin varies considerably with the season and the age of the plant. In bulbils and leaves of young plants, tigogenin is the predominant sapogenin, while in leaves of mature plants, hecogenin is predominant. The tigogenin yield from mature leaves is always low, regardless of origin, and is usually 0.1% of the dry weight. Hecogenin is usually high in plants of Africa and generally low in plants of other places.

Loken and Sollins¹ produced hecogenin from the mixture of pulp and juice of sisal. Beauvoir² obtained sapogenin, particularly hecogenin, from *Agave sisalana* leaves. He separated hecogenin from tigogenin by acetylation and recrystallization. Srinivasulu and Mahapatra³ developed a modified method for extracting pure hecogenin from the dry dewaxed sisal waste. Ernst Wenzler Ger⁴ extracted hecogenin from the sisal sediment; *Agave Vera-cruz* has been planted⁵ in southern India for fibre and as hedges around the fields. On chemical examination of leaves, Srinivasan and Bhatia⁶ have indicated the presence of fructosans and Gedeon and Kincl⁷ have indicated the presence of sapo-

genins such as hecogenin and tigogenin. Gedeon and Kincl⁷, Singh and Pereira⁸, Chakravarti *et al*⁹ and Srinivasulu and Mahapatra³ have isolated hecogenin, tigogenin and other sapogenins from other Indian Agave species.

2. Experimental procedure

In this investigation, hecogenin was extracted from the cake obtained from the hydrolysis of sisal juice of *Agave Vera-cruz*. Various organic solvents like hexane, acetone and toluene were used for extracting hecogenin.

Hydrolysis was carried out with concentrated hydrochloric acid for 4 hours at 70° C (Hydrochloric acid acts as catalyst to break glycosidic bonds). When the hydrolysis was completed, calcium oxide was added to neutralise the acid. The solution was filtered and the cake was washed and dried. Cake contained hecogenin with other hydrolysate. Finally, hecogenin was extracted using a variety of organic solvents in a Soxhlet extractor (Fig. 2) for 22 hours. The solvent was recovered back from the extract to leave substantially pure hecogenin. Hecogenin occurs with tigogenin in most of the Agave species^{10,11} and causes severe problems in separation for the steroid industry¹². It is gratifying to note that *Agave Vera-cruz* collected during various seasons around Bangalore do not appear to contain tigogenin¹³.

About 25-30% of the total volume of the juice is sediment, the major portion of which is hecogenin. Therefore, the yields of cake were established, using (a) the total juice including sediment (Table I), (b) sediment only (Table II) and (c) the juice excluding sediment (Table III) separately using different quantities of hydrochloric acid for hydrolysis.

Table I

Cake content of the total juice including sediment

Sl. No.	Amount of total juice used (c.c.)	Amount of conc. HCl used (c.c.)	Quantity of cake obtained (g)	Conc. HCl used per litre of juice (c.c.)	Cake obtained per litre of total juice (g)
1.	20,000	200	89.61	100	4.48
2.	500	120	4.65	240	9.30
3.	600	125	6.95	208.3	11.58
4.	2,400	500	24.35	208.3	10.54
5.	8,000	1,600	85	200	10.62

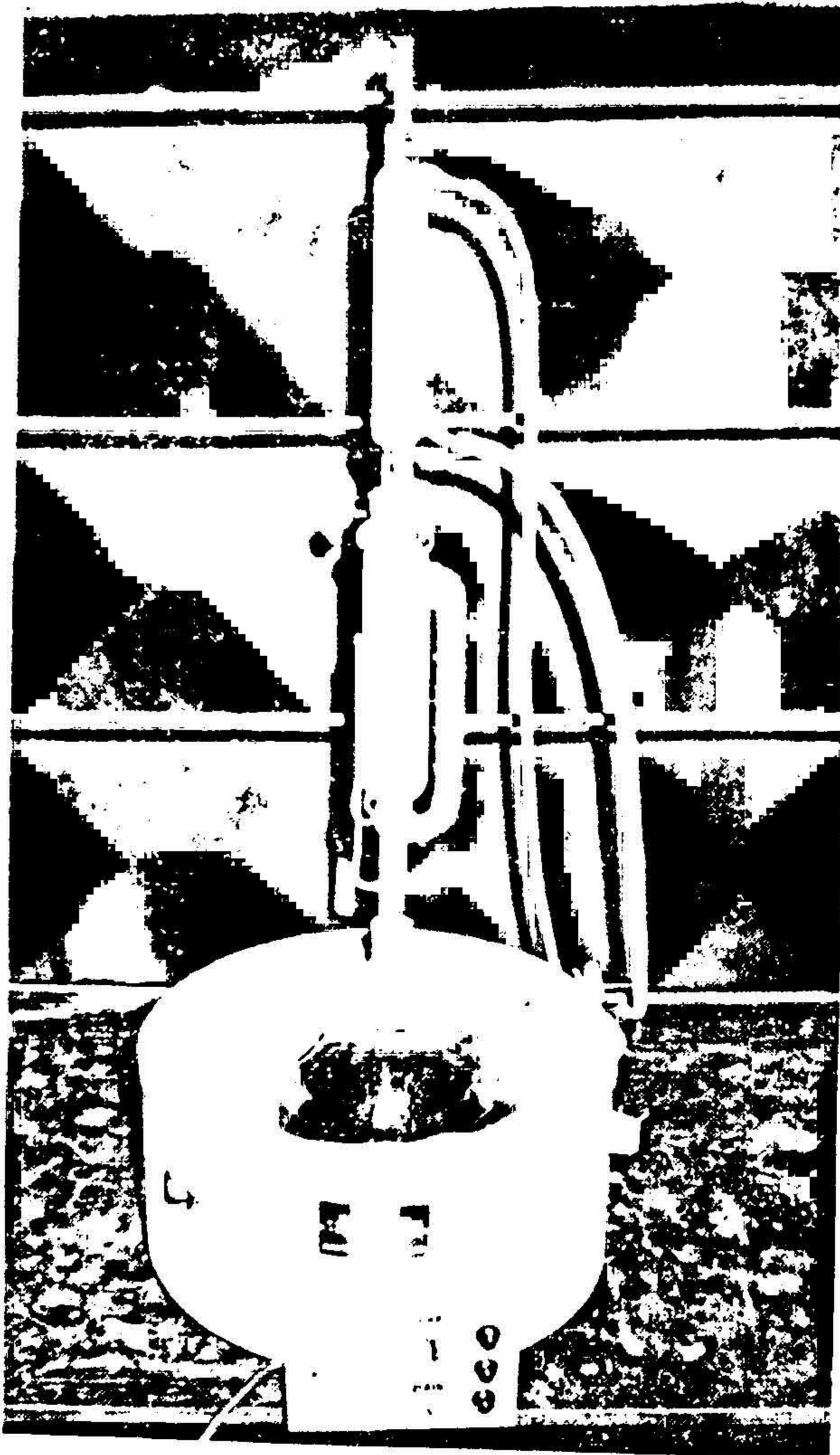


FIG. 2. Soxhlet extractor for extracting hecogenin.

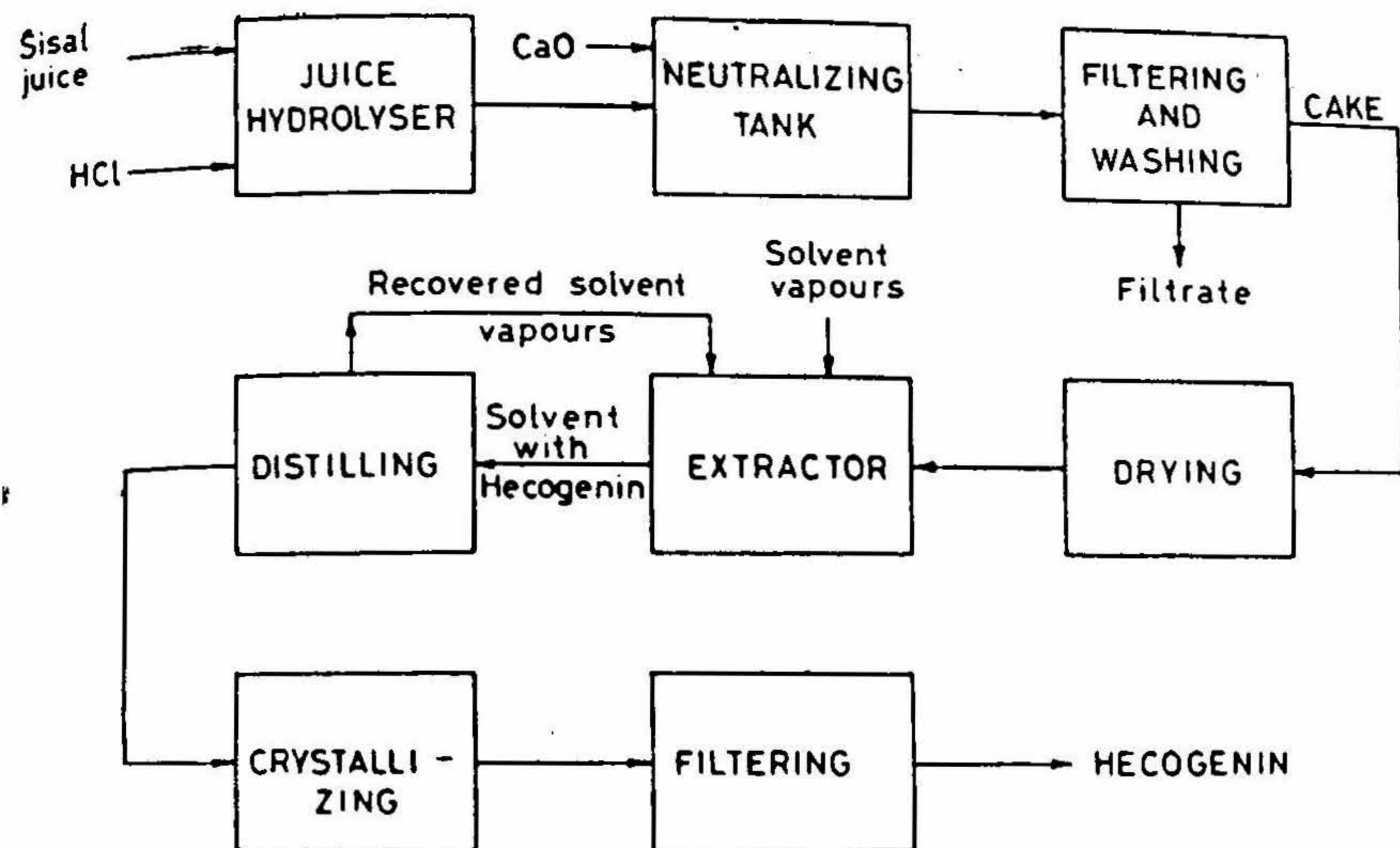


Fig. 3. Flowsheet for producing hecogenin.

Figure 3 shows the various steps for producing hecogenin. The results obtained from the laboratory scale experiments using total juice including sediment and sediment only are shown in tables IV and V respectively.

Table II

Cake content of the sediment

Sl. No.	Amount of juice sediment used (c.c.)	Amount of conc. HCl used (c.c.)	Quantity of cake obtained (g)	Conc. HCl per litre of juice sediment (c.c.)	Cake obtained per litre of juice sediment (g)
1.	1,000	100	26.8631	100	26.8631
2.	1,000	240	26.7850	240	26.7850
3.	12,500	1,250	363.4789	100	29.07

Table III

Cake content of the juice above sediment

Sl. No.	Amount of dilute juice used (c.c.)	Amount of conc. HCl used (c.c.)	Quantity of cake obtained (g)	Conc. HCl used per litre of dilute juice (c.c.)	Cake obtained per litre of dilute juice (g)
1.	1,000	50	5.3546	50	5.3546
2.	1,000	100	7.2288	100	7.2288
3.	1,000	150	6.6885	150	6.6885
4.	1,000	200	5.9518	200	5.9518
5.	1,000	240	7.3657	240	7.3657

Table IV

Yield of hecogenin from sisal juice including sediment

Sl. No.	Quantity of sisal juice used including sediment (c.c.)	Solvent used	Hecogenin obtained (g)	Yield (g/litre of total juice)
1.	500	Hexane	1.231	2.2462
2.	2,400	Hexane	5.0185	2.0910
3.	8,000	Hexane	18.3257	2.2907
4.	600	Hexane	2.7756	4.929
5.	5,000	Acetone	10.2213	2.044

3. Discussion

From tables I, II and III it is clear that the quantity of cake obtained per litre of juice excluding sediment is less in comparison to the quantity of cake obtained per litre of total juice. The maximum amount of cake is obtained from sediment. Further, it may be concluded from tables IV and V that the yield of hecogenin per litre of total

juice is much less than the yield of hecogenin per litre of sediment. In view of these observations it appears that it may be economical to produce hecogenin starting from sediment which contains the major portion of hecogenin. There is no significant variation in the quantity of hecogenin obtained from various solvents (Tables IV and V).

4. Pilot plant for hecogenin

Experimental set-up to produce hecogenin on fairly large scale has been fabricated (Fig. 4). It consists of (a) hydrolyser to treat 35 litres of sisal juice sediment and (b) stainless steel extractor to process cake of about 1,500 g. A double surface condenser has been provided at an angle for the condensation of the solvent vapours to avoid the loss of solvent. The extractor has provisions for : (1) the recirculation of solvent to increase the overall efficiency of extraction and (2) the recovery of solvent after the completion of the extraction. Further work on large scale production of hecogenin is in progress.

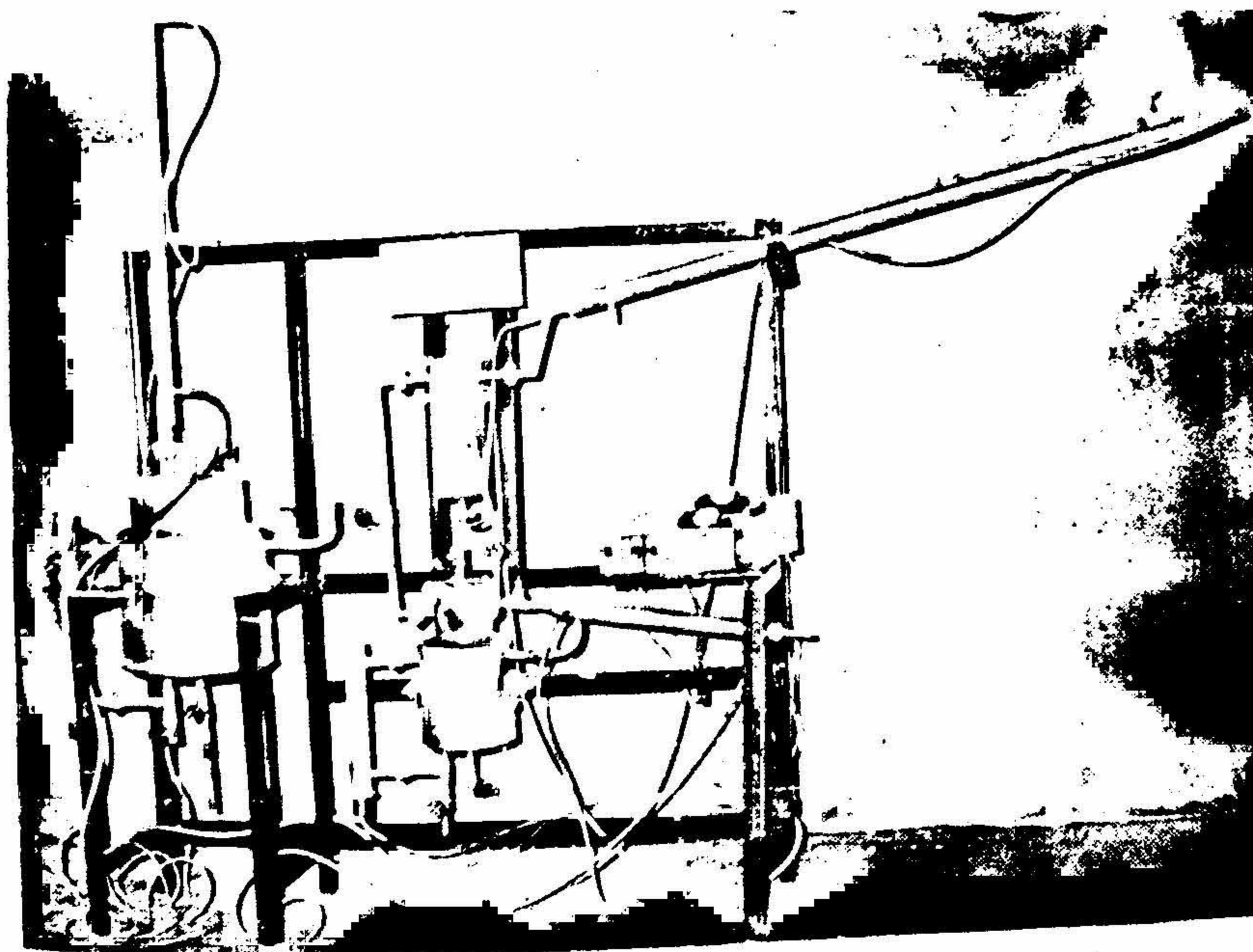


FIG. 4. Pilot plant set-up to produce hecogenin.

Table V

Yield of hecogenin from sediment only

Sl. No.	Quantity of juice sediment used (c.c.)	Solvent used	Hecogenin obtained (g)	Yield (g/litre of juice sediment)
1.	1,000	Acetone	11.8605	11.8605
2.	1,000	Toluene	13.0471	13.0471
3.	1,000	Hexane	12.31	12.31

Acknowledgement

The authors are grateful to Professor A. K. N. Reddy, Secretary, Karnataka State Council for Science and Technology, Bangalore, for his encouragement and helpful suggestions during the various stages of these investigations.

References

1. LOKEN, BJARTE AND SOLLINS, IRVING V. *Chem. Abstr.*, U.S. 3, 510, 400 (Cl. 195-37; C 12 C, C 07C), 05, May 1970, Appl. 26 Apr. 1966, 5 pp.
2. BEAUVOIR, MAX. G. *Chem. Abstr.*, U. S. 3, 981, 867 (Cl. 260-239.55 A; C07 J 17/00), 21 Sep. 1976, Appl. 559,012, 17 Mar. 1975, 7 pp.
3. SRINIVASULU, C. AND MAHAPATRA, S. N. *Res. Ind.*, 1971, 16(3), 183-184.
4. ERNST WENZLER, GER. *Chem. Abstr.*, 1, 235, 844 (Cl c12d), March 9, 1967, Appl. Feb. 6, 1963, 1 p.
5. *The wealth of India*, CSIR, New Delhi, 1948, 1, 40.
6. SRINIVASAN, M. AND BHATIA, I. S. *Biochem. J.*, 1954, 56, 256 and references cited therein.
7. GEDEON, J. AND KINCL, F. A. *Archiv. Pharm.*, 1953, 286, 317.
8. SINGH, H. AND PEREIRA, W. *Indian J. Chem.*, 1964, 2, 297.
9. CHAKRAVARTI, R. N., MITRA, M. N. AND CHAKRAVARTI, D. *Bull. Calcutta School Trop. Med.*, 1958, 6, 60; 1959, 7, 5; 1962, 10, 3.

10. MARKER, R. E.,
WAGNER, R. B.,
RULSHAFFER, P.,
WITTEBECHER, E. L.,
GOLDSMITH, D. P. J. AND
RUOF, C. H. *J. Am. Chem. Soc.*, 1947, 69, 2167.
11. ANTONACCIO, L. D. *Rev. Brazil Quimm.*, 1958, 45, 366.
12. APPELZUEIG, N. *Steroid drugs*, McGraw-Hill, New York, 1962, p. 47.
13. SUBBA RAO, G. S. R. AND
SHYAMA SUNDAR, N. *Indian J. Chem.*, 1974, 12 (4), 429.