PSORALEA CORYLIFOLIA

CHAPTER - 3

BAKUCHIOL AND RELATED COMPOUNDS FROM PSORALEA CORYLIFOLIA LINN.

INTRODUCTION

India with its varied climatic conditions and topography has been considered as "botanical garden of the world" and botanical wealth constitutes more than 2000 types of known medicinal and essential oil bearing plants. Three-fourth of the drugs mentioned in various pharmacopoeia are grown here in their natural state. Indian pharmacopoeia records more than 100 medicinal plants and their preparations and most of them are available in India.

The highly promising results[1,2] with Psoralea corylifolia Linn. in the treatment of leucoderma since early times, and a variety of activity shown by bakuchiol, a constituent from the seed aroused our interest in this plant.

The genus 'Psoralea' which belongs to the family Leguminosae comprises 100-115 species[3] of strongly scented herbs, shrubs and undershrubs, distributed in tropical and subtropical regions.

Psoralea corylifolia Linn* is a common herbaceous weed which grows throughout the whole length and breadth of the plains of India. The seeds are brownish black in colour, about 2-3 mm long and are oblong and flattened. They are hard but not brittle and have a soft skin, known as pericarp. It has an aggreable aromatic odour and a pungent bitterish taste.

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* Its vernacular name are : Sanskrit-Bakuchi, Chanderlekha; Hindi-Babchi, babachi, Bakchi; Punjabi-Babchi; Marathi-Bavachya, Bengali-Bavachi, Latakasturi

CHEMISTRY

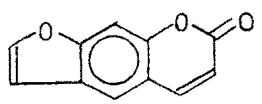
Till date almost 28 new compounds have been reported by various groups from the seeds of P.Corylifolia Linn by various methods.

From the pet.ether extract of seeds, Jois et al.,[4] reported Psoralen (1) and isopsoralen (2). Chakravarti et al., reported[5] psoralidine from the pericarp of the seeds. The structure was then revised (3) by Gupta et al.,[6] Mehta isolated[7] n-heptacosane (4), copaene (5), caryophyllene (6), humulene (7), psoralen (1), isopsoralen (2), caryophyllenol-II (8), caryphyllenol-III (9) and β -sitosterol. A novel meroterpene phenol known as bakuchiol (10), a major constituent of the pet.ether extract of seeds was reported by Sukh Dev and co-workers[8-10].

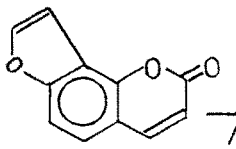
From the solvent ether extract of seeds, Seshadri[11] et al., isolated psoralen, isopsoralen and a phytosterol and synthesised[12] psoralen and isopsoralen. Dhar et al., reported[13,14] isolation of two new chalcones viz. bakuchalcone (11) and isoneobavachalcone (12). Atal et al., reported[15] isolation of triacontane and corylidin (13). Corylifolin (14) and corylifolinin (15) were isolated[16] by Z.G wang-Fang et al.

Chloroform extract of the defatted seeds yielded a series of products colsely related to flavonoids[17] viz. bavachinnin (16) bavachin (17), isobavachin (18), bavachalcone (19) and isobavachalcone (20).

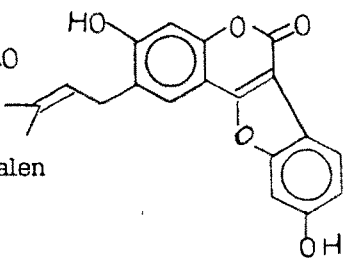
Ethanol extract of the seeds was found to contain a number of compounds.[18-22] These include bavachin (17), psoralidine (3), 4-O-methylbavachalcone, 7-O-methylbavachin, isobavalchalcone (20) and two new compounds namely neobavaisoflavone (21) and bavachromone (22). Psoralen and isopsoralen were obtained as the hydrolysis products of the hydrolysis of defatted



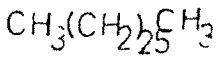
(1) Psoralen



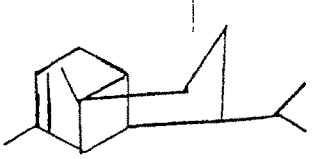
(2) Isopsoralen



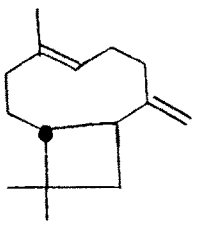
(3) Psoralidine



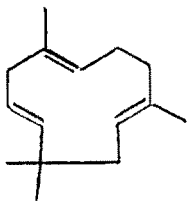
(4) n-Heptacasane



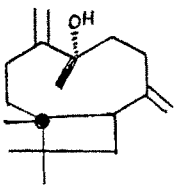
(5) Copaene



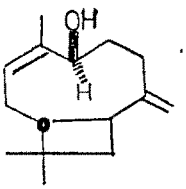
(6) Caryophyllene



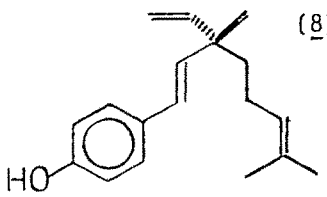
(7) Humulene



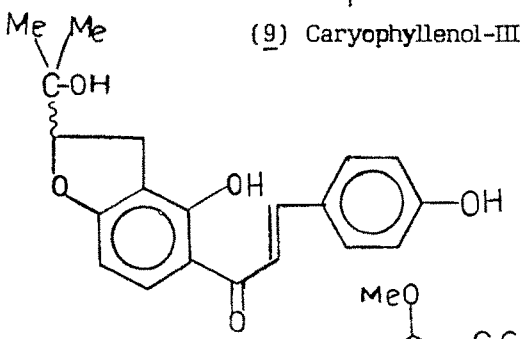
(8) Caryophyllenol-II



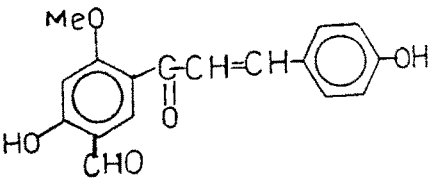
(9) Caryophyllenol-III



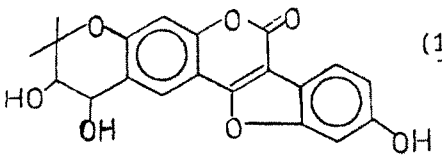
(10) Bakuchiol



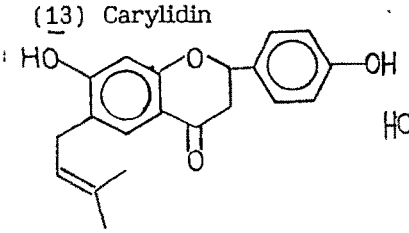
(11) Bakuchalcone



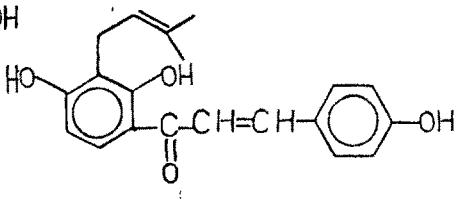
(12)Isoneobavachalcone



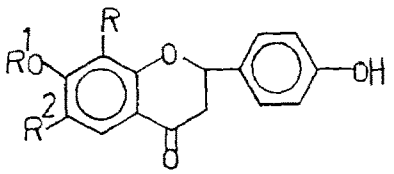
(13) Carylidin



(14) Corylifolin



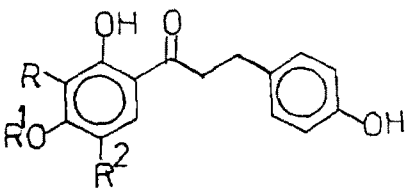
(15) Corylifolinin



(16) R=H, R^1=Me, R^2=$CH_2CH=CMe_2$

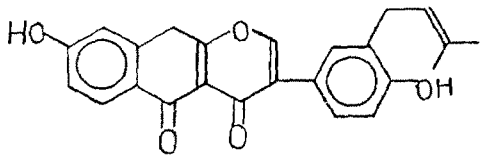
(17) R=R^1=H, R^2=$CH_2CH=CMe_2$

(18) R=$CH_2CH=CMe_2$, R^1=R^2=H

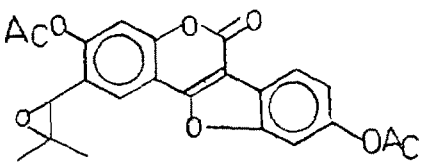


(19) R=H, R^1=Me, R^2=$CH_2CH=C(Me)(Me)$

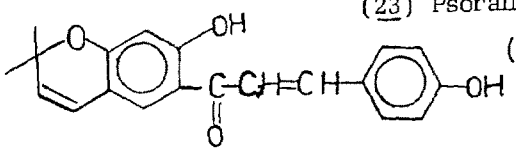
(20) R=$CH_2CH=C(Me)(Me)$, R^1=R^2=H



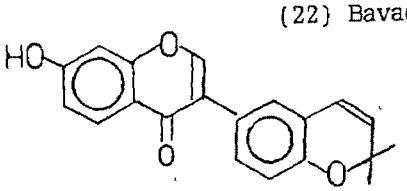
(21) Neobavaisoflavone



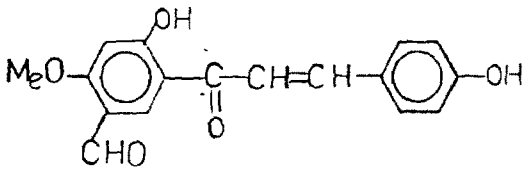
(23) Psoralidine-2-3'-oxidediacetate



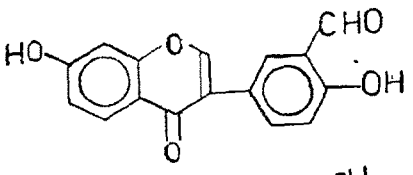
(22) Bavachromone



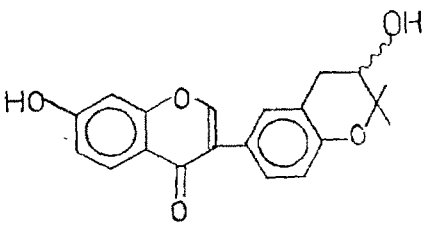
(24) Corylin



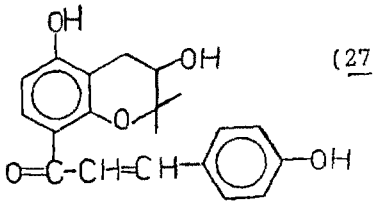
(25) 5'-Formyl-2',4-dihydroxy-4'-methoxy chalcone.



(26) Corylinal



(27) Psoralen



(28) Bavachromanol

seed[23]. Psoralidine-2-3'-oxidediacetate (23) was isolated by Dhar et al.,[24] from the seeds.

The seed oil yielded limonene, ∝-elemene, β-caryophyleneoxide, 4-terpineol, linalool, geranyl acetate, psoralen, isopsoralen and bakuchiol[25].

The pet.ether extract of fruits yielded [26-28] corylin (24), an isoflavone and a formylated chalcone 5'-formyl-2',4-dihydroxy-4'-methoxy chalcone (25), corylinal (26) and neobavaisoflavone (21) and their methyl ethers.

Psoralenol[29] (27) and bavachromanol[30] (28) were isolated from the ether extract of the seeds.

Roots[31] of the plant contained psoralen, isopsoralen, diadzcin, trilaurin and coumasterol.

Ayurvedic Activity

The roots, leaves fruits and seeds of P.corylifolia Linn. are used[32,33] as medicine in Ayurvedic system for many years, as is described in Charaka Samhita, Sushrut Samhita and other books of Ayurveda.

The roots are useful for teeth care and the leaves are for diarrhoea. The fruit is bitter, diuretic, causes biliousness, cures leprosy, skin diseases, "Kapha", "Vata", vomiting, asthama, difficulty in micturition, piles, bronchitis, inflammations, anaemias, improves hair and complexion.

The seeds have been described as hot, sweet bitter, acrid, refrigerant, alterative, laxative, antipyretic, anthelmintic, alexeteric removes "Kapha and Raktapitta", good for heart troubles, asthama, leucoderma, urinary discharges, heals ulcers, skin diseases, scabies. The seeds have been especially recommended in leprosy and are given the name 'KUSHTANASHINI' (leprosy destroyer). In south India, they are used as stomachic and deobstruent and are prescribed in lepra and other cutaneous diseases. Seeds

are prescribed in combination with other drugs for treatment of snake-bite and scorpionsting.

The seeds oil is used in elephantiasis and leucoderma. Dey[34] and Panja[35] have studied the efficacy of oil in treatment of leucoderma.

Pharmacological activity

The essential oil of the seeds of P. corylifolia Linn showed antimicrobial[36,37] and antifungal activity.[37-39]. It has a powerful effect against skin infections caused by streptococci[32]. It has specific effect on the artirioles and subcapillary plexuses, which are dilated so that in this area, plasma is increased and the skin becomes red when melanoblasts are stimulated, leading to pigment formation. The pigment is exuded and diffuses into the decolorised leucodermic patches. The essential oil also stimulated frog's rectus abdominis, the mechanism of action being similar to that of caffiene[40].

From the pet.ether extract of the seeds, a highly potent antistaphylococcal fraction has been isolated[41], the activity of which is stated to be 10 times more than that of chloramphenicol. A high antimicrobial activity[42] has been found to be associated with the pet.ether extract which produces a rise in blood pressure on anaesthetized dogs, causing a stimulation of the intestinal smooth muscle, in vivo and in vitro. Pet.ether extract of the seeds also showed anthelmintic activity against earthworms.[41].

The aqueous and alcoholic extract of the seeds showed antibacterial activity[43] and nematicidal activity[48].

Bavachinine, isolated from seeds showed antiinflammatory activity against carrageenin induced oedema in rats[44]. Antigonadotropic effects of psoralidine were studied and results have been found to be encouraging.[46]

Experiments with the oral administration of furocoumarins present in the seeds, together with local application have shown encouraging results in treatment of leucoderma[1]. Several naturally occurring furocoumarins have altered the response of human skin to UV radiations[45]. Psoralen showed an estrogenic effect in female rabbits but further studies revealed that the effect of estrogen could actually be inhibited by psoralen. Psoralen also seemed to have progesterone like activity on sexually immature rabbits[47]. Psoralen and mixture of psoralen and isopsoralen showed anthelmintic action.[41] Isopsoralen has been reported to have tranquillosedative, anticonvulsant and central muscle relaxant properties in rats, mice and rabbits. It also has mild hypotensive activity in dogs and a non-specific spasmolytic activity on isolated guinea pig ileum, rabbit duodenum and guinea pig uterus[49].

Bakuchiol[8-10] isolated from P. corylifolia Linn seeds showed stronger antimicrobial activity[50], it inhibited gram-positive bacteria,[51-52] including some antibiotic resistant staphylococci, at 1.5 Ml/ml and the dermatophytes tested at 2-20 Mg/ml. It was not active against gram-negative bacteria. Bakuchiol inhibited[53] in vitro growth of Staphylococcus aureus, S. albus and S. citreus. It also exerted high bactericidal and fungicidal activity[54,55] in vitro at 10 Mg/ml and inhibited Staphylococcus aureus after 1-2 mins and Trichophyton mentagrophytes and Microsporum lanosum after 8 mins exposure. The antibiotic activity did not change at pH 5-9 and was not affected by saliva, gastric juices, bile or blood. Bakuchiol also exhibited juvenile hormone (JH) activity[56] more potent than that exhibited by the naturally occurring JH mimic, juvabione[57]. Several synthetic derivatives[58,59] of bakuchiol are found to be much more active than bakuchiol. Bakuchiol exhibited mild CNS activity in mouse at 50 mg/kg iv (intravenously), while cis-methyl ether of bakuchiol caused sedative effect at 100 mg/kg iv[60].

Clinical studies

The oleo-resinous extract of P. corylifolia seeds, containing most of the essential oil was found to be the most effective preparation when applied locally on the patches of leucoderma[61]. Oral administration of mixture of psoralen and isopsoralen with ultra-violet exposure from mercury vapour lamp for 2 to 2.5 minutes gave better results as compared to the external application of crude oil alone in treatment of leucoderma[61].

Local application of an oily mixture of psoralen and isopsoralen in 24 patients of leucoderma for period varying from 1 to 12 months revealed erythema and pigmentation in 21 patients[62]. Application of an ointment of psoralen on patches of leucoderma in 16 patients daily for a period of 1-8 month also produced encouraging results, although the effect was slow.[63] The Ayurvedic preparation having P. corylifolia as the main ingredient showed encouraging results in the treatment of vitiligo.[64] Psoralen, 4,5,8-trimethyl psoralen[65] and 8-methoxy psoralen[66] (oral treatment) in combination with sunlight gave good response in treatment of psoriasis. The action of psoralen on pigment production has been studied in detail by Rashid Ali and Agarwala[67,68].

A mixture of 0.01% bakulchiol and 0.02% pyridinecarboxaldehyde in isopropanol showed encouraging results in the treatment of skin condition such as pimples, comedo, acne and herpes.[69].

From what has been briefly summarised above, it is obvious that Psoralea corylifolia Linn is a premimum plant possessing multifarious virtues exemplified by its therapeutic value as a photosensitising drug. Bakuchiol, psoralen and isopsoralen obtained from the seeds are chief constituents

and are responsible for most of the reported activities of seeds. As a result of studies carried out in W. Germany, bakuchiol, the major constituent of seeds has useful activity for treatment of Psoriasis[70]. In view of this importance, present work was undertaken to develop an expedient and economic process for the isolation of bakuchiol from the seeds. The present chapter describes the development of such a process. In the course of this investigation, a few compounds related to bakuchiol were isolated and identified.

RESULTS AND DISCUSSION

PART - A BAKUCHIOL AND RELATED COMPOUNDS

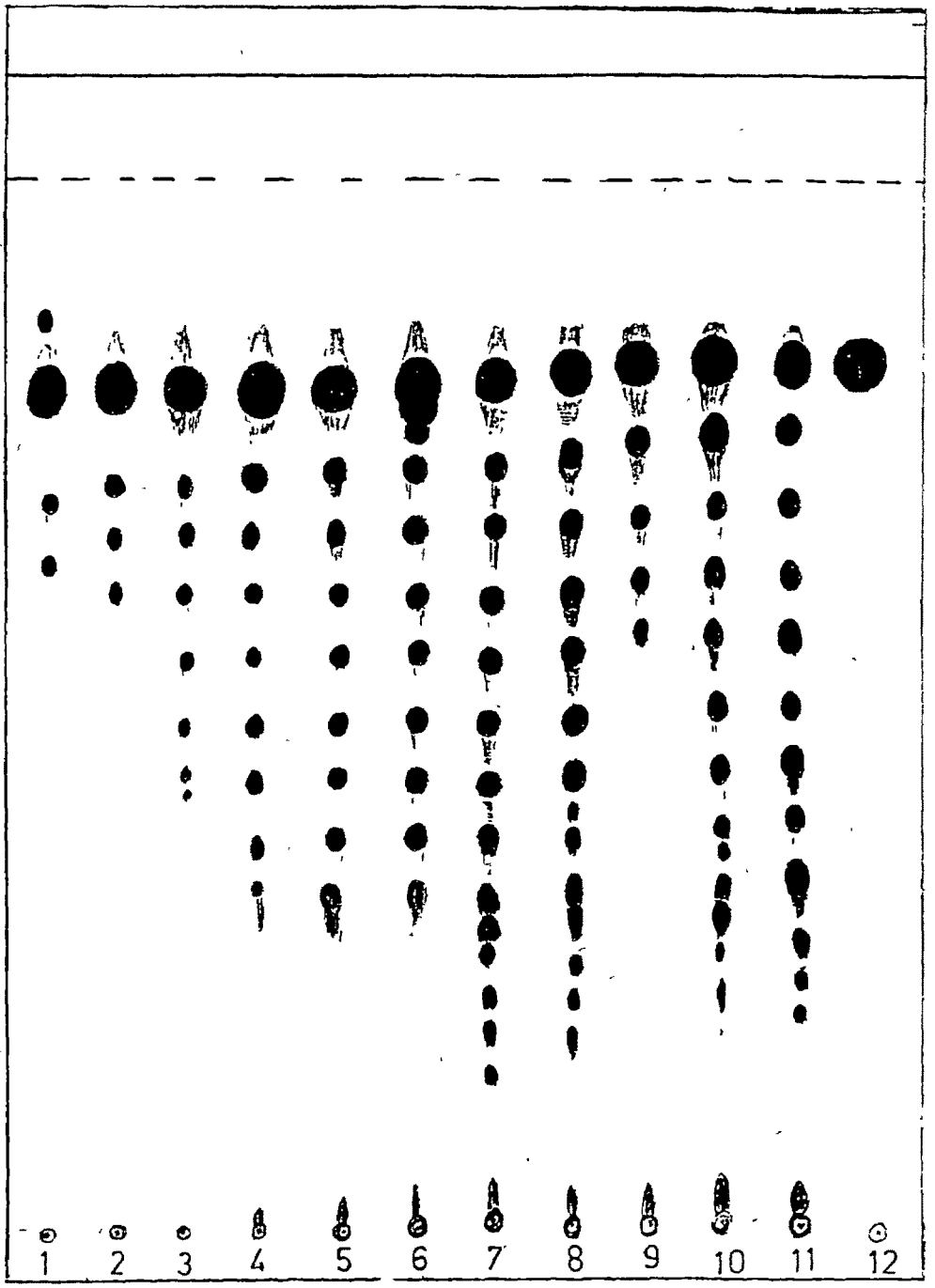
Section - 1 A method for isolation of bakuchiol from Psoralea corylifolia Linn. seeds.

The work was started with a clear intention that the final procedure should be devoid of toxic and expensive solvents and should involve simple and less number of operations. Our first and prime objective was to find out a suitable solvent for extraction.

Whole seeds of Psorelea corylifolia Linn were purchased from market (Baroda) and extracted with different solvents. The seeds were extracted by repeated percolation (extraction) at room temperature. The solvents selected for percolation were pet.ether, solvent ether, benzene and toluene. It was found that pet.ether extraction yielded minimum extract (0.77%) of the seeds, whereas solvent ether extraction yielded maximum extract (12.4%) and toluene extraction yielded (8.4-8.9%) in between the two. Table-1 gives an idea about the extracting capacity of the solvents tried. However, on TLC plate (20% EtOAc in pet.ether, Fig. 1), it was found that pet.ether extract though small in quantity afforded more pure bakuchiol (Fig. 1). Solvent

TABLE - 1 Cold percolation (extraction) of *P. corylifolia* Linn seeds with different solvents.

	Solvent	Wt. of seeds (g)(Time)	Wt. of the extract after each successive extraction (g)(%)			Total wt. of the extract (g) (%)	% of bakuchiol
			1	2	3		
1.	Pet.ether	10.2886 (24 hrs x 3)	0.0434 (0.42)	0.0196 (0.19)	0.0166 (0.16)	0.0796 (0.7736)	
2.	Solvent ether plus pet.ether (1:4)	10.2332 (24 hrs x 3)	0.1810 (1.76)	0.1061 (1.03)	0.0592 (0.57)	0.3463 (3.38)	
3.	Solvent ether plus pet.ether (1:2)	10.0895 (24 hrs x 3)	0.3296 (3.2)	0.119 (1.18)	0.085 (0.84)	0.5351 (5.303)	
4.	Solvent ether plus pet.ether (1:1)	10.1793 (24 hrs x 3)	0.4804 (4.7)	0.1735 (1.7)	0.099 (0.97)	0.7529 (7.3963)	
5.	Solvent ether	200 (24 hrs x 3)				24.94 (12.4)	2.56 %
6.	Benzene	10 (24 hrs x 3)	0.6255 (6.2)	0.1928 (1.9)	0.0484 (0.484)	0.8567 (8.567)	
7.	Benzene plus solvent ether (9:1)	10 (24 hrs x 3)	0.7916 (7.9)	0.3846 (3.8)	0.0805 (0.805)	1.2367 (12.367)	
8.	Toluene	10 (24 hrs x 3)	0.5125 (5.1)	0.2443 (2.4)	0.0913 (0.91)	0.8481 (8.48)	
9.	Toluene	100 (24 hrs x 3)	6.511 (6.5)	1.423 (1.4)	1.048 (1.048)	8.9821 (8.9)	2.53 %



Silica gel - G plate; Solvent system - 20% EtOAc in benzene;
Visualizer - Iodine.

Spots : Cold extraction of seeds with (1) pet.ether (2) solvent ether + pet.ether (1:4) (3) Solvent ether + pet.ether (1:2) (4) solvent ether + pet.ether (1:1) (5) benzene (6) toluene (7) solvent ether (8) solvent ether+ benzene (1:9); Hot extraction of seeds with (9) pet.ether (10) benzene (11) toluene (12) bakuchiol (authentic)

Fig. 1 - Comparative TLC of P. corylifolia Linn seed extracts, extracted with different solvents.

ether extract, owing to the polarity of the solvent, was contaminated with impurities, which were more polar than bakuchiol. Despite of more extract obtained by solvent ether, the solvent can not be an ideal solvent because of the hazards associated with the handling of it in large quantities. To improve the yield of bakuchiol, the seeds were then subjected to hot extraction.

The whole seeds were extracted separately in the sexhlet with hot pet.ether, benzene and toluene continuously for 24 hrs. Results are shown in Table-2. Pet.ether extraction again gave poor yield of the extract (2.5%) but benzene and toluene extraction ($\sim$11%) were comparable (Fig. 1) to that of solvent ether extraction (12%). Toluene was the obvious solvent of choice between toluene and benzene because of the toxic nature of benzene.

Next aim was to find out the exact bakuchiol content in the extract. The three extract viz. solvent ether, toluene (cold extraction) and toluene (hot extraction) were selected for their agreeable yields. All the three extracts were separately chromatographed on silica gel and eluted with pet.ether-benzene with increasing polarity. Crude bakuchiol thus obtained was then distilled to get pure bakuchiol. Results are reported in Table-3, which clearly indicated that the bakuchiol content in all the three extract was nearly same in the range of 2.5-2.6% with respect to the wt. of the seeds.

It is evident from the above findings that toluene is most suitable solvent for extraction because of its comparatively less toxic nature, better recovery and easy handling. Extraction of seeds by cold percolation is a simple and economic process compared to the hot extraction in soxhlet. The seeds (2-15 kg) were repeatedly extracted with toluene by cold percolation.

Table-2 : Hot extraction of P. corylifolia Linn. seeds with different solvents.

No.	Solvent	Wt. of the seeds (g) (time)	Wt. of the extract after each successive extraction (g)(%).			Total Wt. of the extract (g)(%)	% of bakuchiol
			1	2	3		
1.	Benzene	10 (24 hrs)	0.968 (9.6)	0.1191 (1.19)	0.0692 (0.692)	1.1563 (11.56)	
2.	Toluene	100 (24 hrs)	10.2985 (10.2)	1.1872 (1.187)	0.206 (0.2)	11.6917 (11.69)	2.6%
3.	Pet.ether	25 (24 hrs)	0.3368 (1.34)	0.1736 (0.69)	0.1292 (0.516)	0.639 (2.5)	

Table-3 : Bakuchiol content in seed extracted with toluene and solvent ether.

No.	Solvent	Method of extraction	% of seed extract.	% of bakuchiol in extract	% of bakuchiol in seed.
1.	Solvent ether	cold	12.4	20.58	2.56
2.	Toluene	cold	8.9	28.16	2.53
3.	Toluene	hot	11.69	22.24	2.6

Results reported in Table-4 indicate that yield of the extract is in the range of 7-9%.

Isolation of bakuchiol from the toluene extract

After selecting toluene as the solvent for extraction our next target was to isolate bakuchiol from the toluene extract, i.e. to separate pure bakuchiol from the other impurities present in the extract, bearing in mind that the isolation process should not involve any techniques like chromatography or other complex operations. Since all the impurities present in the toluene extract were more polar than bakuchiol, methods were attempted in the belief that the polar impurities can be extracted by using more polar solvent or by counter current distribution between less polar solvent like toluene and more polar solvent or by extracting out bakuchiol from the toluene extract by trituration with non-polar solvent like pet.ether. In each of the process, purity of separated bakuchiol was monitored by TLC. Toluene extract on chromatography afforded 28% bakuchiol indicating that the impurities present in the extract was 72%.

Following were the methods attempted to separate bakuchiol from toluene extract.

(1) Washing of toluene extract with aqueous methanol.

(2) Use of KOH impregnated SiO_2 gel to get rid of polar impurities.

(3) Counter current distribution between [A] toluene and ethylene glycol [B] toluene and propylene glycol.

(4) Trituration of toluene extract with pet.ether.

Exact procedure and results are discussed below :

Table-4 : Cold extraction of Psoralea corylifolia Linn seeds with toluene.

No.	Wt. of the seeds (kg)	Time	Wt. of the extract after each successive extraction			Total wt. of the extract (g) (%)
			1 (%)	2 (%)	3 (%)	
1.	2	24hrsx3	120.4 (6.0)	32.2 (1.6)	17.2 (0.85)	169.4 (8.45)
2.	2	24hrsx3	137 (6.85)	37.5 (1.87)	2.1 (0.105)	176.6 (8.53)
3.	10	24hrsx3	492.2 (4.92)	122.9 (1.229)	93.5 (0.935)	708.6 (7.086)
4.	10	24hrsx3	630.4 (6.304)	132.2 (1.34)	35.4 (0.354)	800.0 (8.0)
5.	14.95	24hrsx3	1022.99 (6.82)	182.99 (1.22)	143.99 (0.96)	1350.0 (9.0)

(1) Washing of toluene extract with aqueous methanol (20%).

Pure bakuchiol is completely soluble in toluene and benzene but has only 28% solubility in aq. methanol (20%). In order to remove the polar impurities, the toluene extract was dissolved in benzene and repeatedly extracted with aq. methanol (20%). After usual work-up 77.8% was obtained as benzene soluble portion and 22.2% was obtained as aq. methanol soluble portion. However, TLC of both the fractions showed a poor purification of bakuchiol. The method was rejected.

(2) Use of KOH impregnated silica gel.

This method was opted assuming that the more polar impurities might get adsorbed on deactivated SiO_2 gel impregnated with alkali thus rendering purer bakuchiol. 20% KOH impregnated SiO_2 gel was prepared in such a manner that it retained at least 18% water. The toluene extract was dissolved in toluene (large excess). To it was added KOH impregnated silica gel in portions and after each addition, it was stirred for five minutes and spotted on TLC. Even after addition of alkali impregnated SiO_2 gel (five times the wt. of the extract), impurities were not removed. Obviously, this method could not be an adequate method for isolating bakuchiol.

(3) Counter curent distribution between toluene and ethylene glycol.

The principle behind the method was that of the partition coefficient of compounds in two different immiscible solvents.[71] The solvents used were toluene and ethylene glycol. The toluene extract was dissolved in excess of toluene and successively extracted with ethylene glycol. Each of the ethylene glycol extracts was reextracted with toluene (counter current). TLC after solvent removal indicated that bakuchiol was distributed in first and second toluene fractions and it was contaminated with polar impurities. The two

fractions were combined and redissolved in toluene and the process was repeated once again. Pure bakuchiol was again distributed in first and second fractions, which were combined. The light yellow viscous material obtained after removal of solvent, was distilled at 160-65°/0.9 mm to get pure bakuchiol The distilled yield of bakuchiol was 25.3% against 28% expected. However, the the method is complicated because the number of operations involved.

When ethylene glycol was replaced by propylene glycol, bakuchiol obtained was of inferior quality.

When distribution between toluene and ethylene glycol containing 2,5 % KOH was tried, the separation was very poor.

(4) Trituration of toluene extract with pet.ether.

In this process, toluene extract after complete removal of solvent was chilled to 3-5° and the viscous mass formed was triturated with chilled (~ 3°) pet.ether. This process was repeated till the pet.ether insoluble portion showed a very faint spot of bakuchiol on TLC. Combined pet.ether soluble portions showed much pure bakuchiol (TLC).

As this method gave promising results, further experiments were carried out from 2 gm -1.3 kg scale. The results are represented in Table-5. The pet.ether extract (enriched in bakuchiol) was 50-54% of toluene extract, clearly indicating that bakuchiol obtained by this process was contaminated with ~ 50% impurities. Final attempts were then made to achieve TLC pure (~ 90%) bakuchiol from this enriched portion. As chromatography can not be commercially feasible process, other method like counter current distribution or passing through a bed of some adsorbent or hydrodistillation had to be tried.

Counter current distribution of pet.ether soluble portion between toluene and ethylene glycol yielded TLC pure bakuchiol (22.3%, expected

Table-5 : Trituration of tulene extract with pet.ether.

No.	Wt. of the toluene extract (g)	Vol. of pet.ether	Wt. of the pet.ether - soluble portion (g) (%).	
1.	2.0973	50 ml x 4	1.049	(50.01)
2.	9.8271	50 ml x 4 25 ml x 4	5.316	(54.09)
3.	22.4495	50 ml x 12 100 ml x 5	11.8078	(52.59)
4.	54.9	50 ml x 10 100 ml x 10	30.01	(54.66)
5.	95.0	50 ml x 10	51.4236	(54.13)
6.	708.6	500 ml x 4 250 ml x 9	350.0	(49.39)
7.	800.0	400 ml x 14	430.0	(53.75)
8.	1350.0	400 ml x 23	675.0	(50.0)

28%). But the process was again complicated and involved number of extractions.

Very poor yield (3.5%) of bakuchiol was obtained, when bakuchiol enriched portion was hydrodistilled. Even co-distillation of pet.ether soluble portion with ethylene glycol followed by extraction of bakuchiol from distillate with toluene, after usual work-up and distillation furnished very poor yield of bakuchiol (4.5%).

The last option was to pass the enriched material through a bed of a adsorbent like neutral alumina. For this purpose neutral alumina of various grades were prepared. The bakuchiol enriched fraction (containing at least 50% pure bakuchiol) was adsorbed on it and filled in a column and eluted with solvent like pet.ether followed by benzene or toluene.

Alumina of grade IIa, IIb and IIIa were prepared and the separation tried using them. Out of the three grades tried, alumina-IIb in the ratio of 1:7 (i.e. 1 g enriched fraction vs 7 g alumina-IIb), when eluted with hot toluene in the soxhlet gave the best yield of TLC pure bakuchiol (26-27%). Results are reported in Table-6.

On the basis of the above methods the final procedure was established for the isolation of bakuchiol from whole seeds of Psoralea corylifolia Linn. The method is simple and convenient, devoid of the technique like column chromatography, involves minimum operations and can be a commercially viable process.

Final procedure

Whole seeds of Psoralea corylifolia Linn (~ 10 kg) were percolated with toluene (10 l) at room temperature (24 hrsx3). All the three extract

Table - 6 : Isolation of bakuchiol using alumina.

No.	Wt. of the toluene extract (g)	Wt. of the pet.ether soluble portion (g)(%)	Alumina (g) (grade)/ ratio+		Solvent used for extraction or elution	Wt. of the crude bakuchiol	Wt. of the distilled bakuchiol (g)(%)*
1.	9.8271	5.3106 (54.04)	14 1	(IIa)/ : 2.6	Pet.ether and benzene	3.67	0.7342 (20.0)
2.	22.4494	11.8078 (52.59)	28 1	(IIa)/ : 2.37	Pet.ether and benzene	7.6935	4.5983 (20.48)
3.	95.0	51.4236 (54.13)	350 1	(IIIa)/ : 6.8	Pet.ether and toluene.	30.70	22.286 (23.45)
4.	54.9	30.0129 (54.668)	156 1	(IIb)/ : 5	Pet.ether and toluene.	12.1622	10.0797 (18.35)
5.	33.11	20.00 (60.4)	140 1	(IIb)/ : 7	Pet.ether and toluene	13.086	9.0759 (27.4)
6.	71.51	43.2 (60.4)	302.4 1	(IIb)/ : 7	Toluene	24.9133	18.6651 (26.1)

+ Ratio of alumina used is with respect to pet.ether soluble portion of seeds extract.

* Yield of bakuchiol is with respect to toluene extract of the seed. Expected yield is 28% on the basis of column chromatography.

were combined, which after solvent removal furnished dark viscous gum (7.086%). It was chilled (~ 3°) in an ice bath and triturated with cold pet.ether (~3°) repeatedly till the extract showed a very faint spot of bakuchiol on TLC (250mlx7). Pet.ether was removed on water bath and last traces of solvent were removed under reduced pressure to get bakuchiol enriched portion as dark brown liquid (43.39% of toluene extract). It was dissolved in pet.ether (3 L) and adsorbed on neutral alumina grade-IIb (2.45 kg) on rotary evaporator for 2 hrs (initially at 40° for 1 hr and then at 80° for 1 hr). The impregnated alumina was filled in a thimble and extracted continuously in the soxhlet with toluene (5 lit) for 15 hrs (till the extracting solvent showed a very faint spot of bakuchiol on TLC). Toluene was stripped off under reduced pressure on rotary evaporator to yield crude bakuchiol (2.12%), which was fractionally distilled to get TLC pure bakuchiol (160.25 g, 1.6%).

The above procedure was confirmed by repeatition and results are reported in Table-7.

Section-2 A method for analysis of bakuchiol.

Having been able to get a suitable method of isolation, our efforts were then directed to find out the proper time of harvesting when the seeds have maximum bakuchiol content. Since most of the herbal drugs are stored for long time, we were interested in finding whether or not the bakuchiol content remains the same during storage.

In order to carry out above study, it was essential to find out convenient and accurate method of analysis for the estimation of bakuchiol in seeds. Column chromatography and gas chromatography are suitable methods

Table-7 : Extraction of bakuchiol from the seeds of Psoralea corylifolia Linn.

Expt. No.	Wt. of the seeds (kg)	Wt. of the toluene extract (g) (%)	Wt. of the pet.ether soluble portion (g) (%)	Wt. of the crude bakuchiol (g) (%)	Wt. of the distilled bakuchiol (g) (%)
1	10	708.6 (7.086)	350.0 (49.39)	212.14 (2.12)	160.25 (1.6)
2	10	800.0 (8.0)	430.0 (53.7)	250.0 (2.5)	180.0 (1.8)
3	14.95	1350.0 (9.0)	675.0 (50.0)	352.0 (2.3)	240.0 (1.6)

of analysis for most types of compounds, hence it was decided to go for column chromatography and gas chromatography.

Column Chromatography

The toluene extract of the seeds was column chromatographed on SiO_2 gel and eluted with pet.ether followed by pet.ether-benzene and finally column was washed with methanol. The chromatography was monitored by TLC. The fractions containing bakuchiol were combined and distilled to get TLC pure bakuchiol as light yellow viscous oil. Bakuchiol content in the toluene extract estimated by column chromatography was found to be 31.78% and in the seed was 2.53%.

Despite its reliability, the method cannot be used as a routine analytical method because it is tedious and time consuming. Also, the bakuchiol yield is based on weight of the distilled product but the exact purity of the distilled bakuchiol may not be 100%.

Gas Chromatography

Alcohols and phenols (of high molecular weight) because of their polar nature or high boiling point do not come out of most of GC columns or decompose during analysis, but their volatile derivatives like esters and ethers are stable and have been used for analysis by GC.[72]

The toluene extract of the seeds was distilled at 140-250°/0.5 mm and the distillate (54.8%) was silylated with trimethylsilychloride (TMSC) and hexamethyldisilazane (HMDS) in pyridine.[73]. Residue obtained after usual work-up was subjected to GC analysis on 10% OV-4 at 190°. The GC purity of the trimethylsilyl ether was 82.8%. On the basis of GC analysis, bakuchiol content in the toluene extract was 42.41% (expected 31.78%) and in the

seed was 3.37% (expected 2.53%). It was guessed, that this difference may be because of the non-volatile impurities present in the extract, which may not have come out of the GC column and thus showing high percentage of bakuchiol than expected.

Gas Chromatography with internal standard

To overcome the above problem, it was decided to use pure compound as an internal standard[74] for GC analysis. Since bakuchiol is a phenolic compound, the compounds selected for internal standard were either alcohols or phenols which could be silylated alongwith bakuchiol. TMS derivatives of number of compounds were prepared and analysed by GC under same conditions. Table-8 shows the retention time of the compounds tried. Cetyl alcohol (RT 3.41 mins, Fig. 2) was most satisfactory internal standard for bakuchiol (RT 7.83 mins.)

The area correction factor (A.C.F.) for the column was calculated as follows :

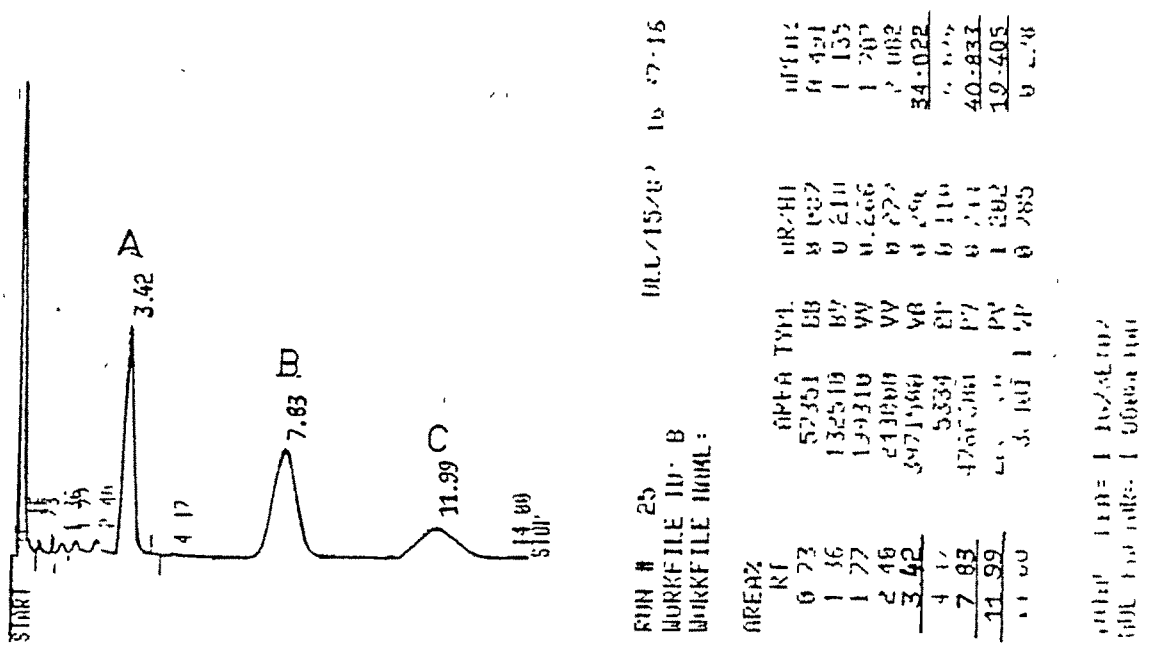
Accurately weighed (100-200 mg) cetyl alcohol of known purity (97.36%) was mixed with nearly the same quantity of accurately weighed bakuchiol of known purity (99.3%). Four different mixtures were prepared and silylated separately. The silylated mixture was analysed by GC.

$$\text{A.C.F.} = \frac{\text{Wt. of bakuchiol x Area of internal std. x purity of the internal std.}}{\text{Wt. of internal std. x Area of bakuchiol x purity of bakuchiol}}$$

According to this method the percentage of bakuchiol in toluene extract was 33.61% (expected 31.78%) and 2.67% (expected 2.53%) in the seeds.

Table-8 : List of the internal standard tried and its retention time (GC), compared to bakuchiol.

Sr. No.	Compound	Retention time (mins).
	Bakuchiol	7.83
1.	Thymol	0.6
2.	α-Napthol	1.24
3.	β-Napthol	1.43
4.	Eugenol	0.93
5.	Octanol	0.38
6.	Decanol	0.56
7.	Cetyl alcohol	3.41
8.	Stearyl alcohol	7.03



TMS ether of A - cetyl alcohol, B - bakuchiol, C - unknown compound (or derivative of bakuchiol).

Column 10% OV-4, Temp. 190°C, Detector - FID.

Fig. 2 - GC analysis of Psoralea corylifolia Linn seeds extract with internal standard (cetyl alcohol).

As percentage of bakuchiol obtained by this method was very close to the expected value, this method of estimation was accepted as a reliable method and used for further studies.

Section - 3 Maturity of seeds and bakuchiol content

After succeeding in getting the suitable method of analysis, we started to work on our main objective i.e. to find out the variation in the content of bakuchiol with ageing of the seeds. For this purpose Psoralea corylifolia Linn was cultivated in our nursery and two types of immatured seeds were collected in beginning of December and directly extracted with toluene. Matured seeds were collected in beginning of January and stored in a porous cotton bag at room temp. From the stored seeds, 50-100 gm of the seeds were regularly extracted at an interval of 30 days for six months and last lot of the seeds were extracted at the end of one year (i.e. at the end of December).

For general procedure, from each lot of seeds, 5-10 g (accurately weighed) of the seeds were heated at 120° for 8 hrs and the moisture content was found out by loss in weight. The stored seeds (50-100 g) were extracted (every month) with toluene and extract after removal of solvent was stored in refrigerator under nitrogen. All the extracts were analysed after a year, for which accurately weighed extract was distilled and the distillate was subjected to analysis by GC with internal standard (in duplicate). Results are reported in Table-9.

It was evident from the results obtained that there was a gradual increase in yield of toluene extract in first four month after harvesting (4.18% $\longrightarrow$ 8.38%) and then there was gradual decrease in last eight months,

Table - 9 : Estimation of Bakuchiol in P. corylifolia Linn seed, extracted at the interval of every 30 days for a year.

Type of seeds (month of extraction)	Wt. of the seeds (g) (% of moisture)	Wt. of the seeds on dry wt. basis (g)	Wt. of the total extract (g) (% on dry wt. basis)	Wt. of the extract taken for distillation (g)	Wt. of the distillate (g) (%)	Wt. of the bakuchiol in distillate (g) (%)	% of bakuchiol in total extract.	% of bakuchiol in seed (on dry wt. basis)
Immatured (green)	147.0 (36.4)	94.8	3.68 (3.92)	1.4716	0.8066 (54.8)	0.5315 (65.9)	36.11	1.425 contained psoralen & isopsoralen.
Immatured (black)	137.0 (36.65)	86.78	2.55 (2.94)	1.19	0.6238 (52.42)	0.4918 (78.85)	41.32	1.215
Just matured (black) (January)	90.0 (10.57)	80.32	3.37 (4.18)	0.8184	0.5301 (64.7)	0.4811 (90.76)	58.78	2.47
Matured (February)	100 (5.98)	94.02	5.594 (5.94)	1.311	0.8319 (63.45)	0.6739 (81.01)	51.4	3.05
Matured (March)	50.0 (5.6)	47.2	3.1031 (6.574)	0.4824	0.2846 (59.0)	0.1923 (67.59)	39.87	2.6214
Matured (April)	50.0 (4.7)	47.64	3.99 (8.38)	0.9733	0.5651 (58.0)	0.2967 (52.51)	30.48	2.554
Matured (May)	50.0 (4.99)	47.5	3.75 (7.89)	0.7855	0.4238 (53.9)	0.3321 (78.37)	42.28	3.339
Matured (June)	50.0 (8.3)	45.98	3.1025 (6.7)	0.8676	0.5362 (61.0)	0.3453 (64.41)	39.8	2.844
Matured (July)	50.0 (7.0)	46.5	3.0707 (6.6)	0.6353	0.3987 (62.75)	0.1834 (46.0)	28.86	1.906
Matured (December last week)	100.0 (7.6)	92.4	4.33 (4.68)	0.7829	0.5054 (64.55)	0.4170 (82.5)	53.26	2.49

but percentage of bakuchiol in the extract was highest in the month of February and May and lowest in July. This was an abnormal variation of bakuchiol content observed in the toluene extract.

An interesting observation was that, the percentage of toluene extract was more in the green and immature seeds than in the black and just matured seed. When toluene extract of the green seeds was distilled a white compound crystallized out in the distillate. The distillate was diluted with pet.ether and the white crystalline compound was separated by filtration. It was found to be a mixture of psoralen and isopsoralen in the ratio of 87.5%: 12.5 % (by GC, 10% OV-4 190°). These two compounds are mostly present inside the seeds and very little in the pericarp.[75] Presence of psoralen and isopsoralen in the toluene extract of the prematured seeds can be explained like this. The soft pericarp of the prematured seeds must have allowed the solvent to penetrate through the seed and let psoralen and isopsoralen be extracted out. The soft seeds were found broken during the process of extraction, which could be another reason for their extraction. Extraction of psoralen and isopsoralen by toluene thus increased the yield of toluene extract. In case of matured and hard seeds, it was not at all observed. Thus, it is not advisable to harvest prematured seeds.

There can be three following factors responsible for abnormal variation of bakuchiol content in the toluene extract.

(1) Incomplete distillation of bakuchiol while distilling the toluene extract.

(2) Thermal decomposition of bakuchiol during distillation.

(3) Some bio-chemical changes taking place during the long storage of the extract.

First two possibilities were checked by avoiding distillation of the crude toluene extract. Crude extract of the seeds extracted in the month of July, May and February were silylated and the TMS ether were analysed by GC with internal standard. The results obtained were very close to the results obtained by analysis of the distilled extract. It was concluded that neither of the two factors were responsible for the variation.

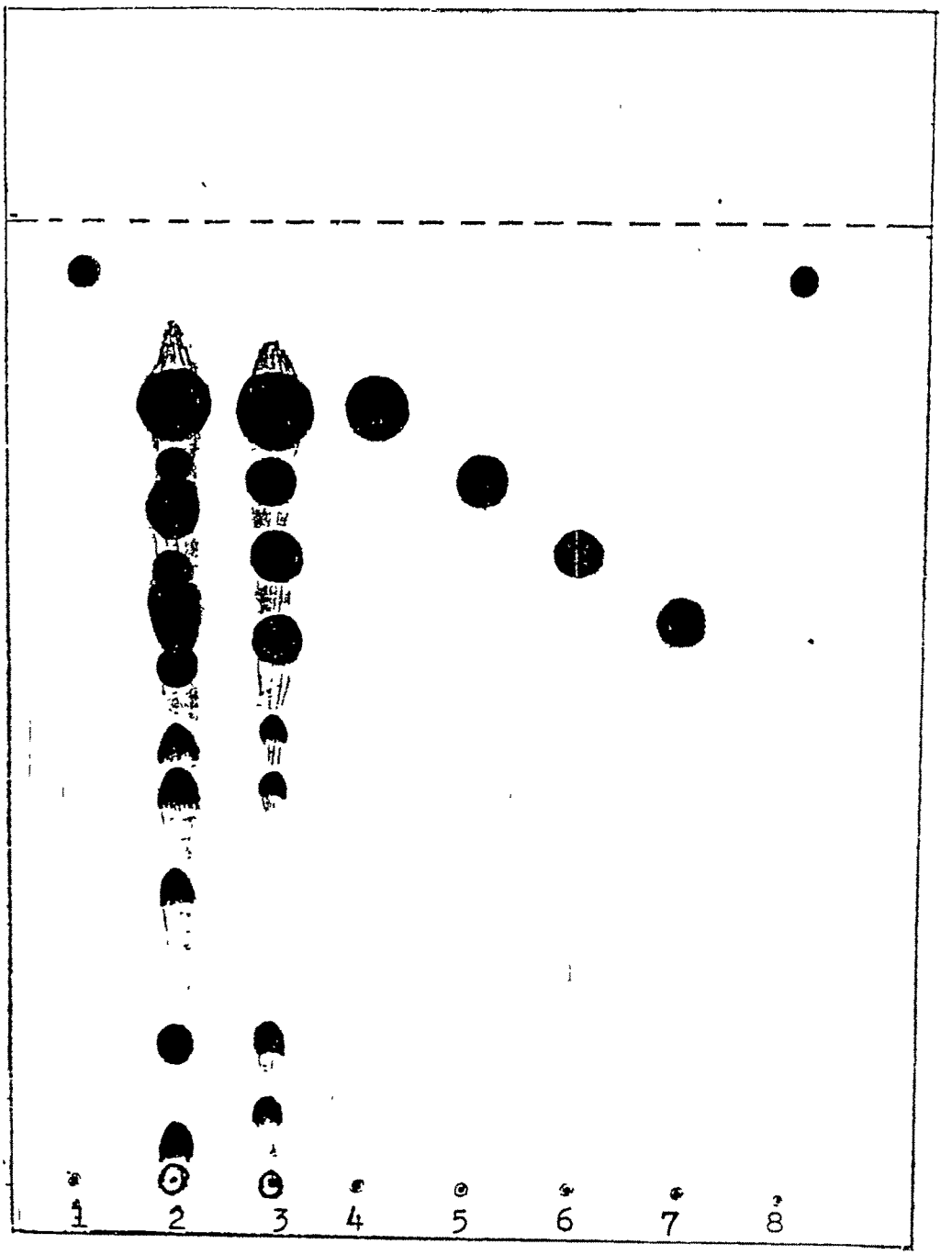
There was a third possibility that due to some bio-chemical changes, bakuchiol gets converted into some other compound which may be a derivative of bakuchiol. The transformation might be taking place either during the storage of the extract or directly in the seeds. During analysis (GC) of TMS ether of the extract with internal standard, we observed an extra peak having retention time (~ 12 min. Fig. 2) higher than bakuchiol. When percentage of bakuchiol was recalculated by adding the percentage of compound having higher retention time to the percentage of bakuchiol calculated earlier (Table-10A), the variation in yield was found to be normal. Bakuchiol content was maximum in the matured and 3-4 months old seeds and decreased gradually with time. This was in support to our assumption that there was a bio-chemical transformation of bakuchiol into its derivatives.

It was then essential to isolate and identify the unknown compound. Fractional distillation of the extract failed to isolate it, hence the extract was subjected to chromatography on chromatotron using benzene-ethylacetate as eluent. The chromatography was monitored by TLC. Four compounds were isolated.

Bakuchiol (10) was isolated in the earlier fractions and was confirmed by comparison with the authentic sampele (TLC Rf 0.81, Fig. 3; GC TMS

Table-10 A : Comparative percentage of bakuchiol estimated earlier and recalculated (by adding percentage of bakuchiol estimated earlier in percentage of compound having higher retention time than bakuchiol.

Type of seeds	% of bakuchiol (estimated earlier)	% of bakuchiol recalculated
Gree seeds (prematured)	1.42	1.45
Black seeds (just matured)	1.215	1.31
Matured (January)	2.47	2.64
Matured (February)	3.05	3.50
Matured (March)	2.62	3.50
Matured (April)	2.55	3.97
Matured (May)	3.33	3.94
Matured (June)	2.84	3.59
Matured (July)	1.90	2.88
Matured (December)	2.49	2.79



Silica gel - G plate; Solvent system 20% EtOAc in benzene. Spray reagent- 0.5% anisaldehyde in 2% H_2SO_4 in CH_3COOH.

(1) Dye (Sudan III) (2) Crude extract (3) distilled extract (4) Bakuchiol (5) 6,7-Epoxy bakuchiol (synthetic) (6) Δ^{7},6-Hydroxy bakuchiol (7) Δ^{5},7-Hydroxy bakuchiol (8) Dye (Sudan III).

Fig. 3 : TLC of toluene extract of P. corylifolia Linn seeds.

ether Rt 7.83 min, Fig. 4, IR, NMR) The structure of bakuchiol was further confirmed by ^{13}CMR.

IR (neat): OH 3350, 1230 cm^{-1}; an aromatic ring 1610, 1512 cm^{-1}; $CH=CH_2$ 1625, 995, 910 cm^{-1}; trans CH=CH 970 cm^{-1}.

PMR (CCl_4)δ: C_3-Me(3H, S, 1.18 ppm), C_7-Me's (3H, S, 1.56 and 3H, S, 1.64 ppm), p-disubstituted benzene ring (4H, AA'BB' apparent quartet, centered at 6.89 ppm J_{AB} = 8.5 Hz), $\Delta^{11,12}$(2H, AB-type centered at 6.05 ppm, J_{AB}= 16 Hz), $\Delta^{1,2}$(3H, m, ABC type located between 4.6 and 5.93 ppm), $\Delta^{6,7}$(H, ~ 4.9 ppm).

^{13}CMR (Fig. 5) see Table-10B.

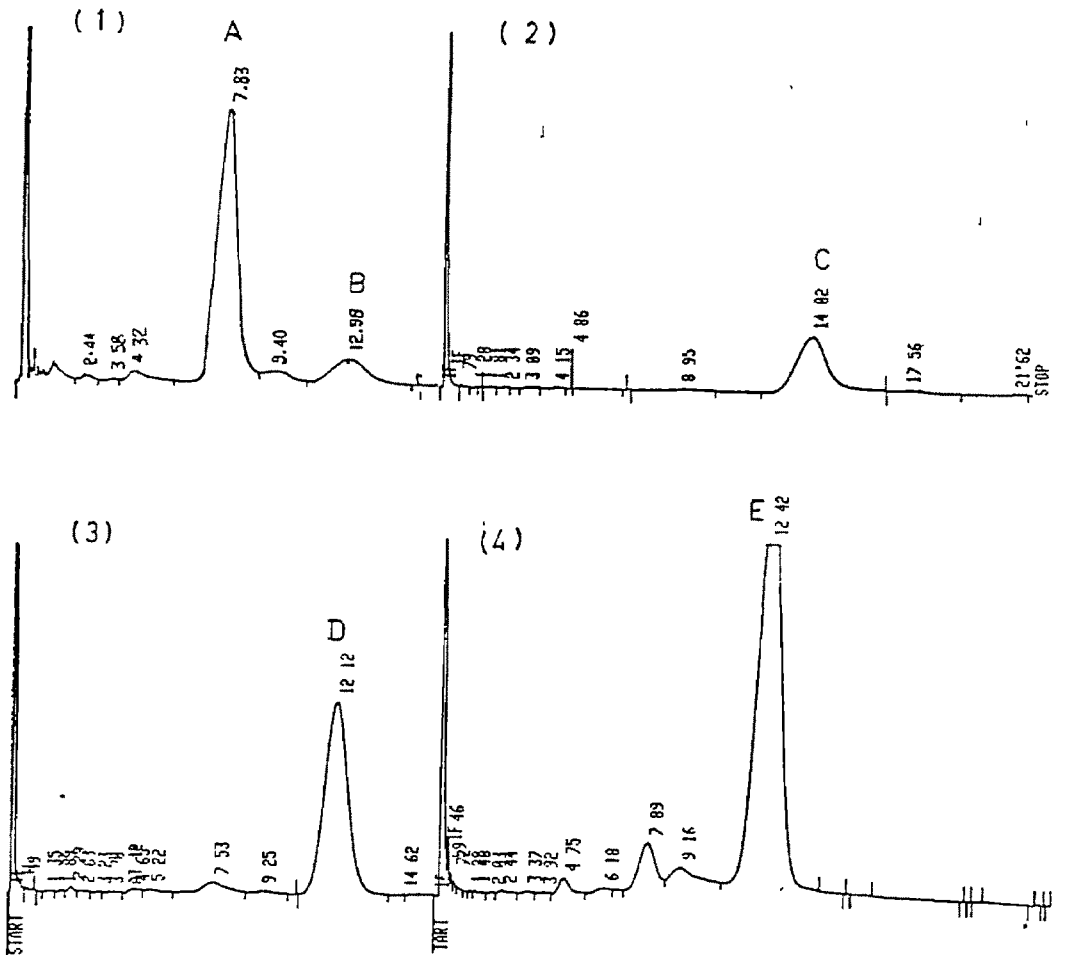
Section - 4 New Constituents

Compound A : A new compound (A) (95 mg, ~ 75% TLC pure, Rf 0.74) was obtained as a colourless viscous oil. Further purification of this compound could not be carried out because the compound exhibited unstable nature during distillation and column chromatography. The compound was found to be identical with 6,7-epoxybakuchiol (29) by comparison of IR and NMR with that of authentic sample.[76] Later the compound was synthesized from bakuchiol and compared with the isolated compound.

IR(neat,Fig. 6) : OH 3420 cm^{-1}, an aromatic ring 1605, 1535 cm^{-1}, trans CH=CH 970 cm^{-1}, $CH=CH_2$ 1505, 910, 1000 cm^{-1}.

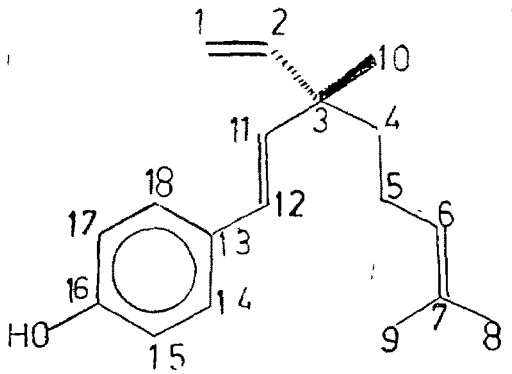
PMR (CCl_4, Fig. 7)δ: C_3-Me (3H, S, 1.13 ppm), C_7-Me's (3H, S, 1.22 and 3H, S, 1.27 ppm), p-disubstituted benzene ring (4H, AA'BB' apparent quartet centered at 6.88 ppm, J_{AB}=9 Hz), $\Delta^{11,12}$(2H, AB-type centered at 6.01 ppm, J_{AB}=16 Hz), $\Delta^{1,2}$(3H, m, ABC-type located between 4.8 and 5.9 ppm).

^{13}CMR ($CDCl_3$, Fig. 8) see Table-10B.

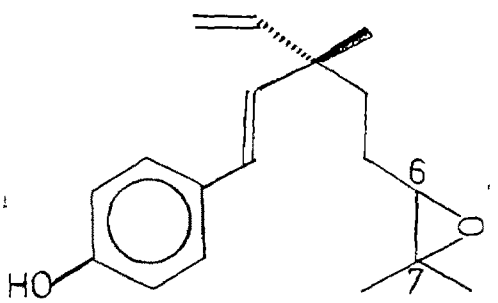


(1) TMS ether of Psoralea corylifolia Linn seed, extract in the month of July. A-bakuchiol (Rt 7.83 mins), B-unknown compound (Rt 12.98 mins).
(2) TMS ether of 6,7-Epoxybakuchiol (Rt 14.02 mins) synthetic.
(3) TMS ether of Δ^{7},6-Hydroxybakuchiol (Rt 12.12 mins).
(4) TMS ether of Δ^{5},7-Hydroxybakuchiol (Rt 12.42 mins).
Column - 10% OV-4, Temp. 190°C, Detector-FID.

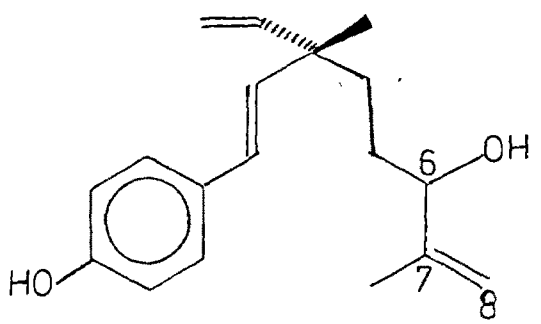
Fig. 4 : GC analysis of Bakuchiol and its derivatives.



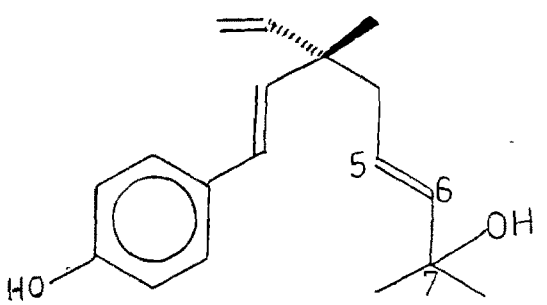
(10) Bakuchiol .



(29) 6,7-Epoxybakuchiol



(30) Δ^{7},6-Hydroxybakuchiol



(31) Δ^{5},7-Hydroxybakuchiol

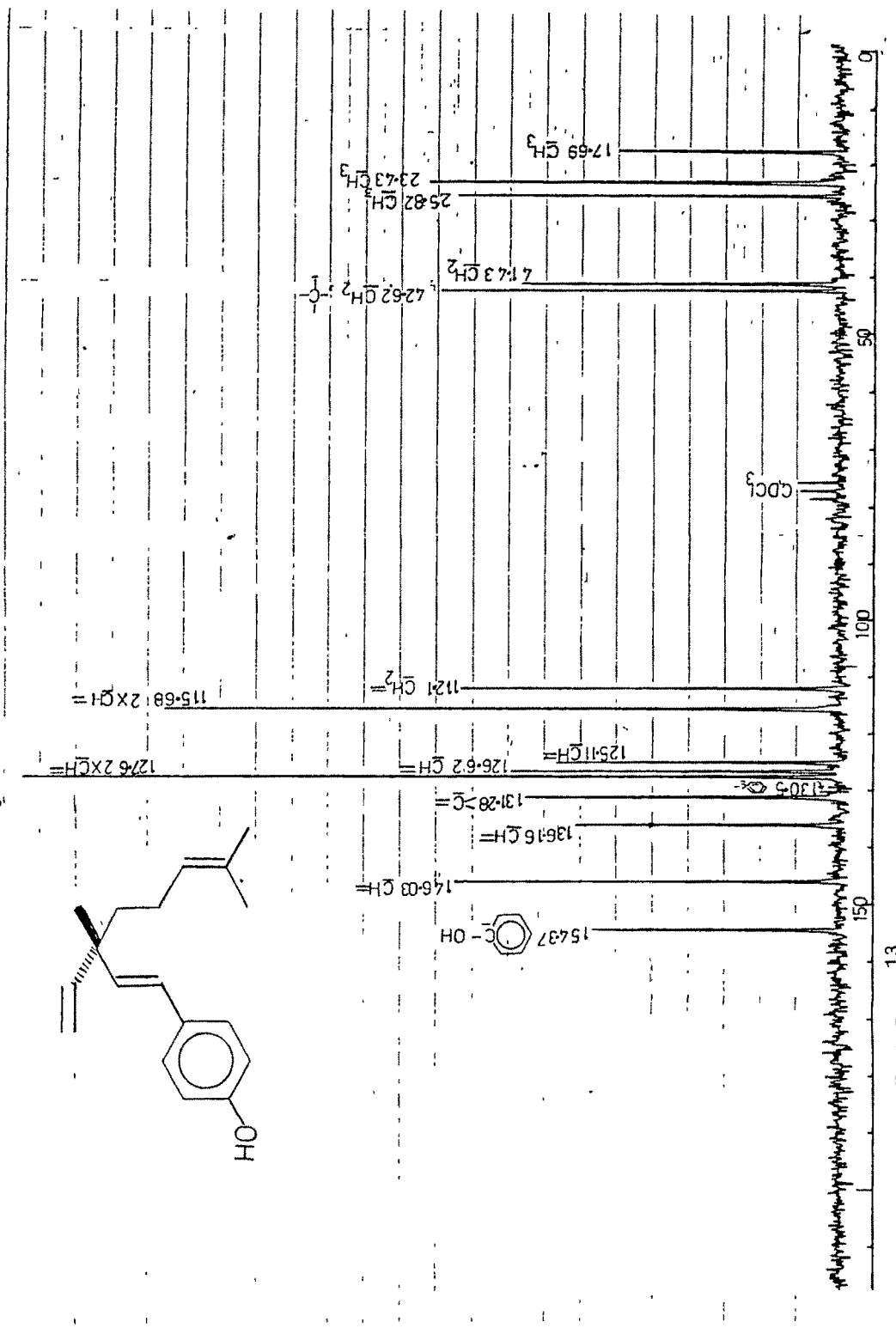


FIG.5 - ^{13}C NMR SPECTRUM OF BAKUCHIOL.

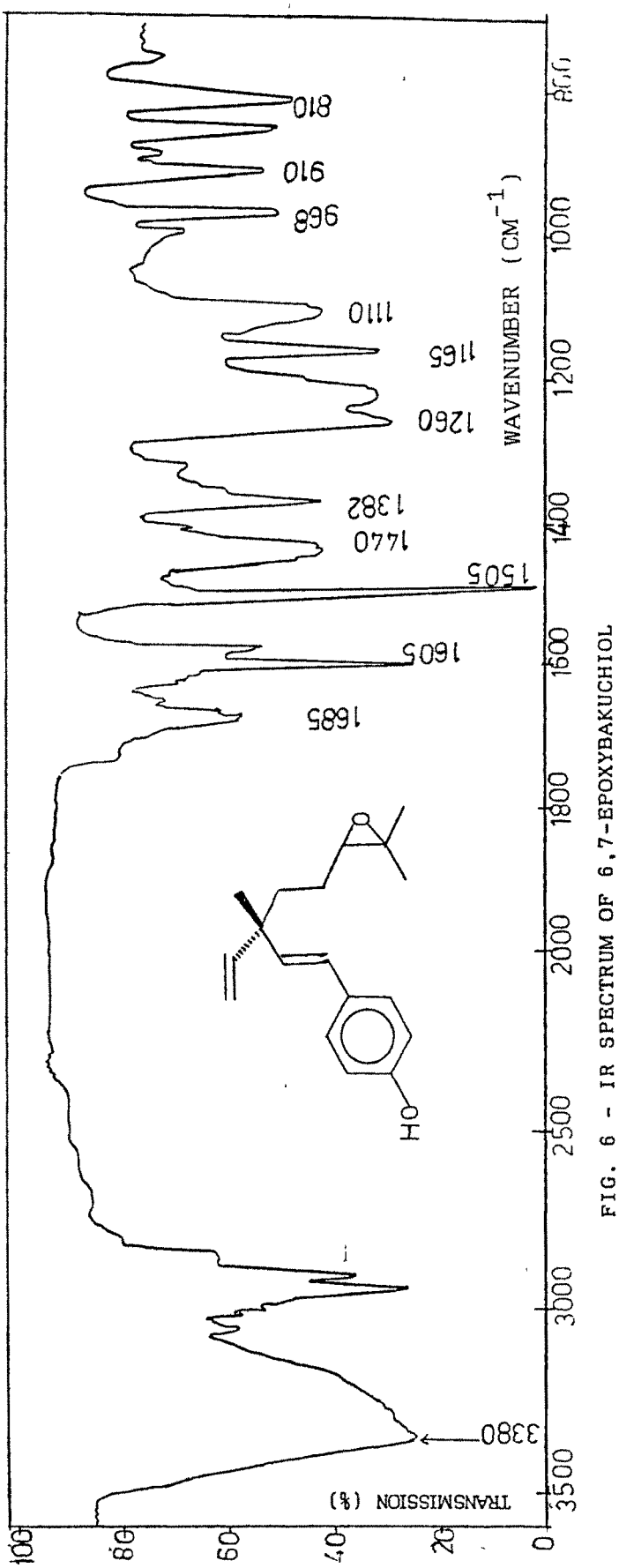


FIG. 6 - IR SPECTRUM OF 6,7-EPOXYBAKUCHIOL

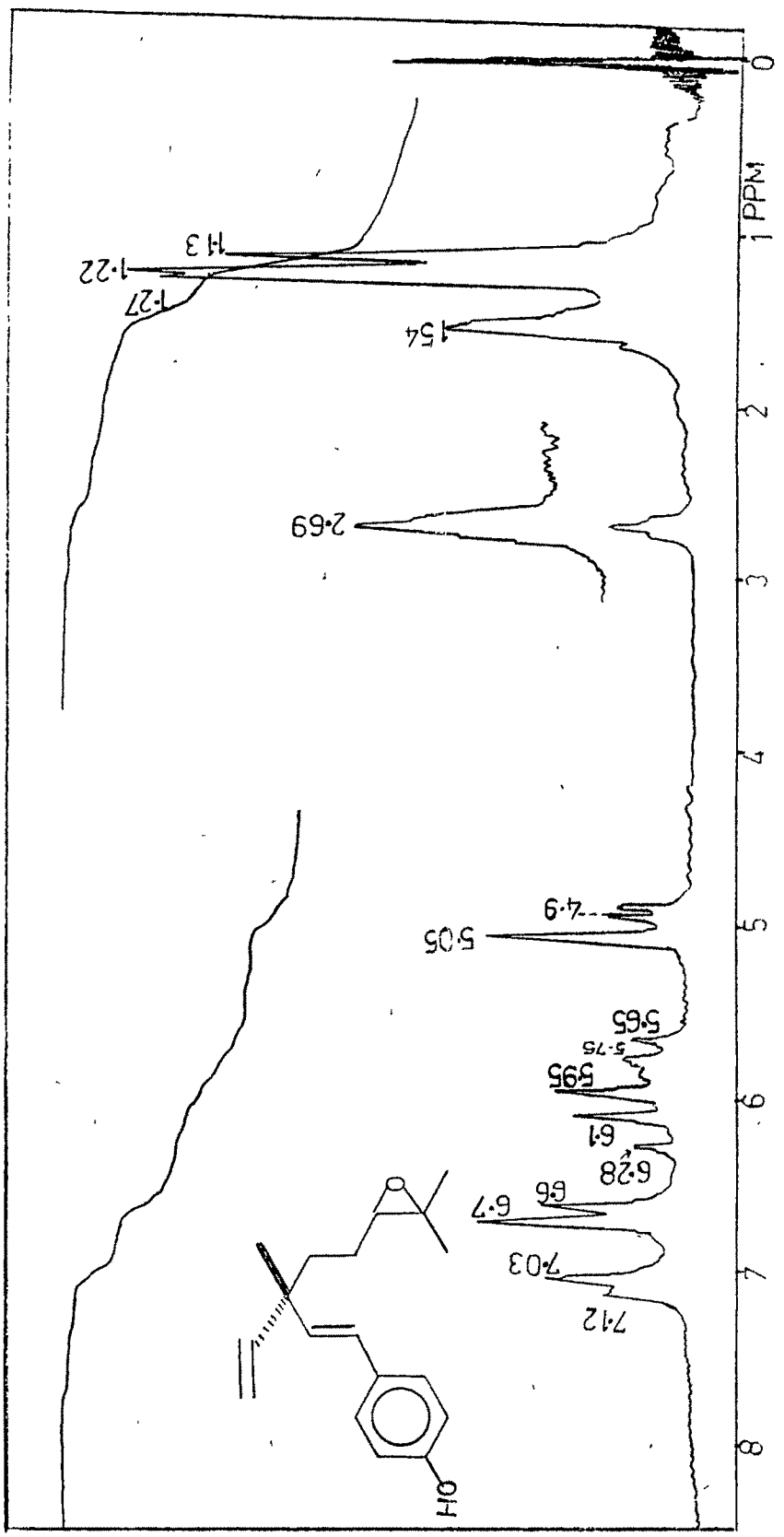


FIG. 7 - NMR SPECTRUM OF 6,7-EPOXYBAKUCHIOL.

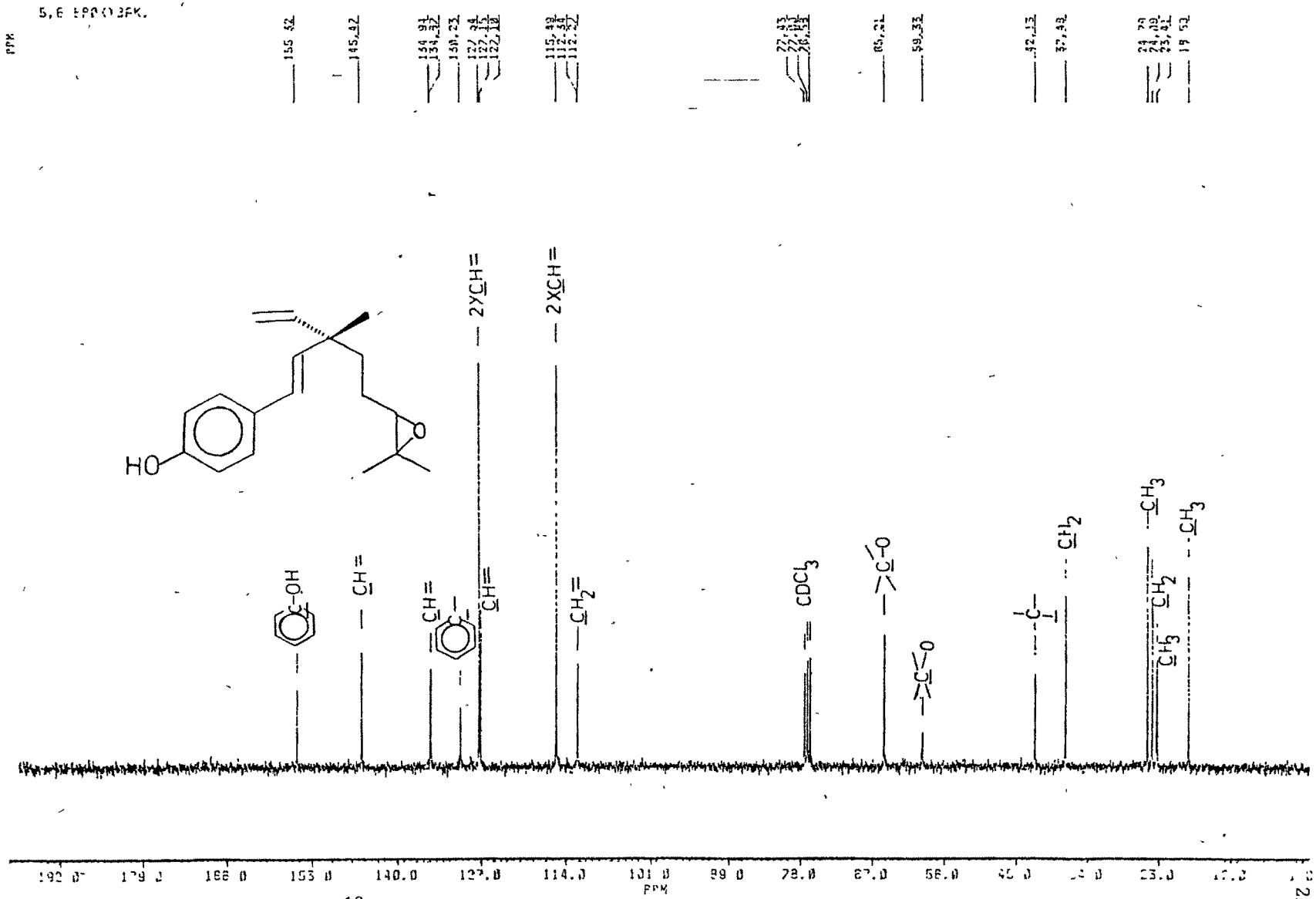


FIG. 8 - ^{13}C NMR SPECTRUM OF 6,7-EPOXYBAKUCHIOL.

Compound B :

Compound - B (85 mg) was eluted in later fractions. It was further purified on chromatotron, followed by distillation at 185-200°(bath temp)/0.5 mm to furnish 60 mg TLC pure colourless liquid (Rf 0.65, Fig. 3), GC of its TMS ether showed 90% purity (Rt 12.12 min, Fig. 4). The compound was identified as Δ^{7},6-hydroxybakuchiol (30) by comparison of IR and NMR (Fig. (Figs. 9,10 and 11) with that of the authentic compound. The structure was further confirmed by ^{13}CMR (Fig. 11)

IR (neat, Fig. 9) : OH 3420 cm^{-1}, an aromatic ring 1610, 1515 cm^{-1}, trans CH=CH 980 cm^{-1}, $CH=CH_2$ 910, 1005 cm^{-1}.

PMR δ($CDCl_3$, Fig. 10): C_3-Me (3H, S, 1.13 ppm), C_7-Me (3H, S, 1.67 ppm) p-disubstituted benzene ring (4H, AA'BB' apparent quartet centered as 6.88, J_{AB}=9Hz), $\Delta^{11,12}$(2H, AB-type centered at 6.02 ppm, J_{AB}=16 Hz), $\Delta^{1,2}$(3H, m, ABC-type located between 4.7 and 5.93), $\Delta^{7,8}$(2H, located between 4.7 and 5.1 ppm).

^{13}CMR ($CDCl_3$ Fig. 11) see Table 10B.

Compound C :

Compound - C (25 mg) on further purification on chromatotron furnished 16 mg TLC pure (Rt 0.59, Fig. 3) colourless liquid. GC of its TMS ether showed 89% purity (Rf 12.42 min, Fig. 4). It was identified as Δ^{5},7-hydroxy-bakuchiol (31) by comparison of IR, NMR (Fig. 12, 13) with the authentic compound. The structure was further confirmed by ^{13}CMR.

IR (neat, Fig. 12) : OH 3420 cm^{-1}, an aromatic ring 1510, 1610 cm^{-1}, trans CH=CH 972 cm^{-1}, $CH=CH_2$ 915, 1100 cm^{-1}.

PMR δ($CDCl_3$, Fig. 13): C_3-Me (3H, S, 1.15 ppm), C_7-Me's (6H, S, 1.29 ppm), p-disubstituted benzene ring (4H, AA'BB' apparent quartet centered at 6.89 ppm,

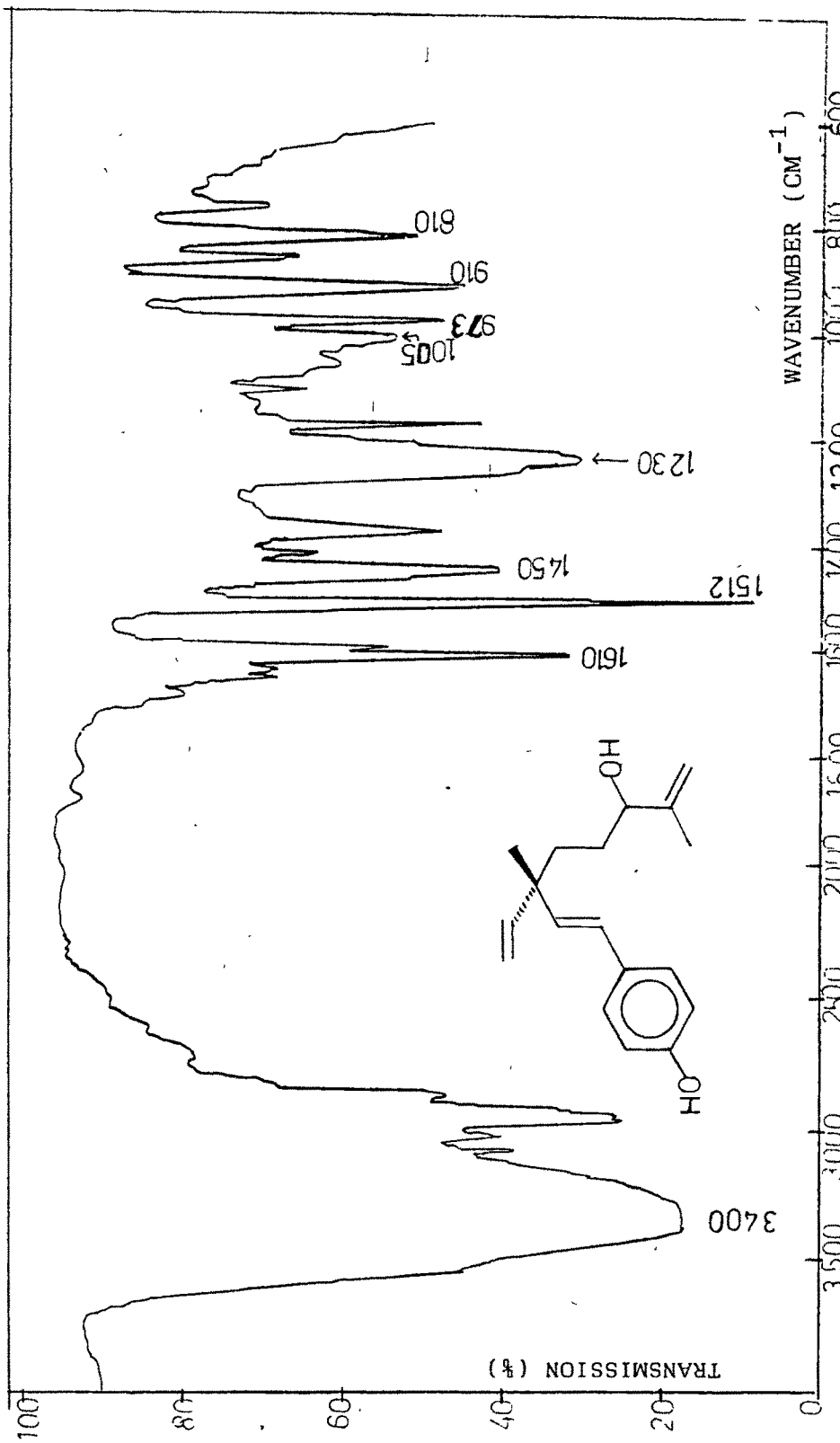


FIG. 9 - IR SPECTRUM OF Δ^7,6-HYDROXYBAKUCHIOL

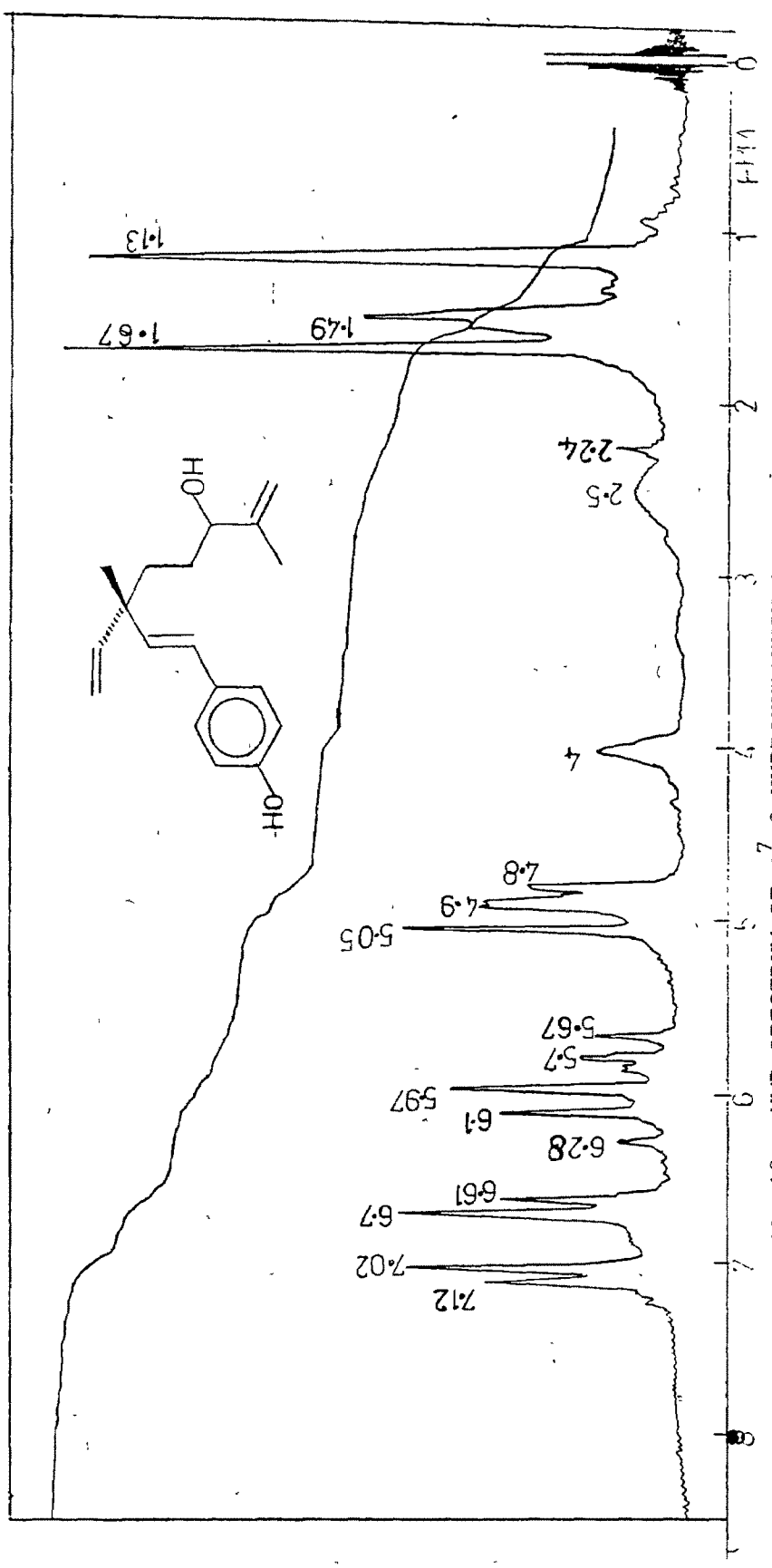


FIG. 10 - NMR SPECTRUM OF Δ^7,6-HYDROXYBAKUCHIOL.

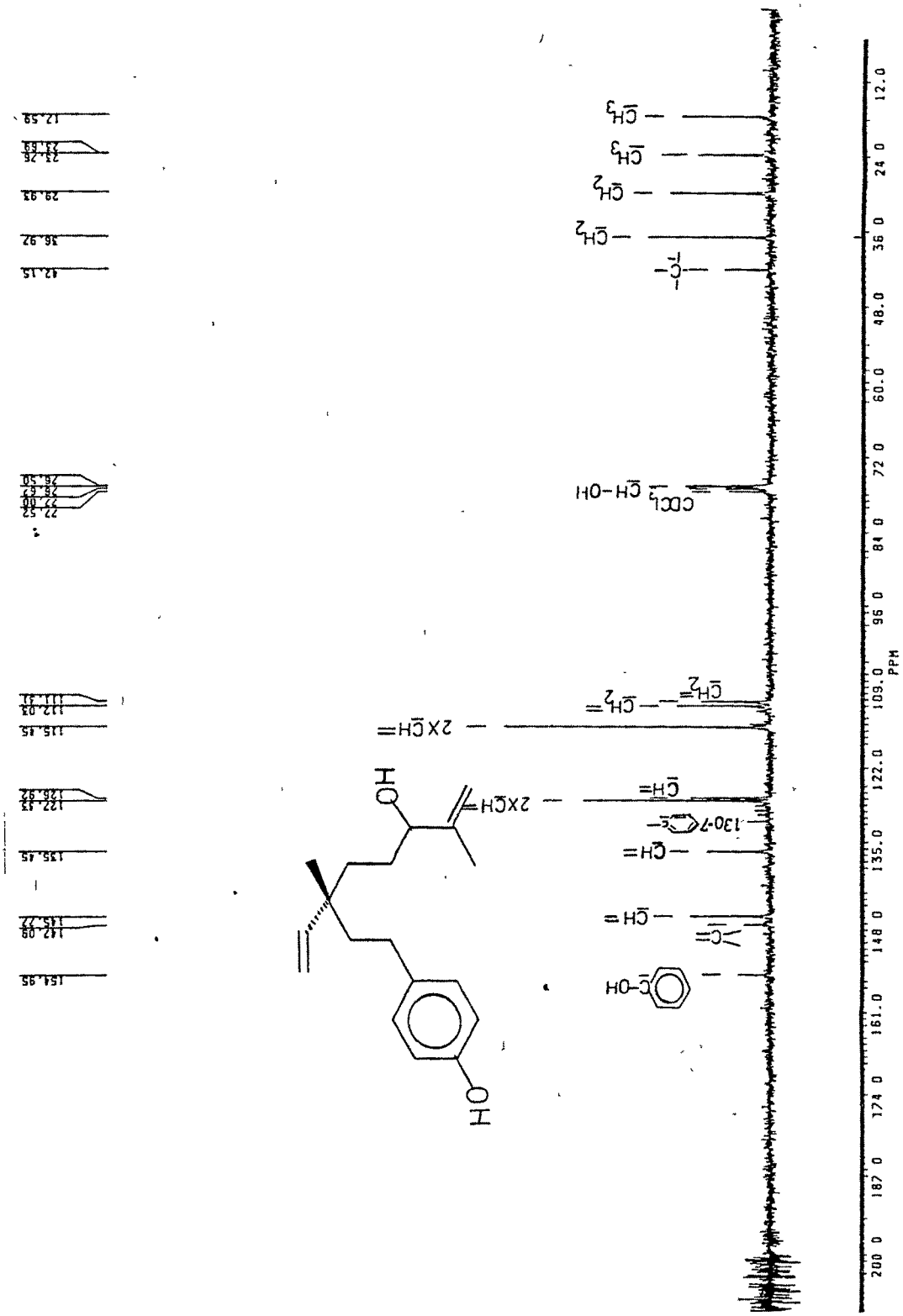


FIG. 11 - ^{13}C NMR SPECTRUM OF Δ^{7},6-HYDROXYBAKUCHIOL.

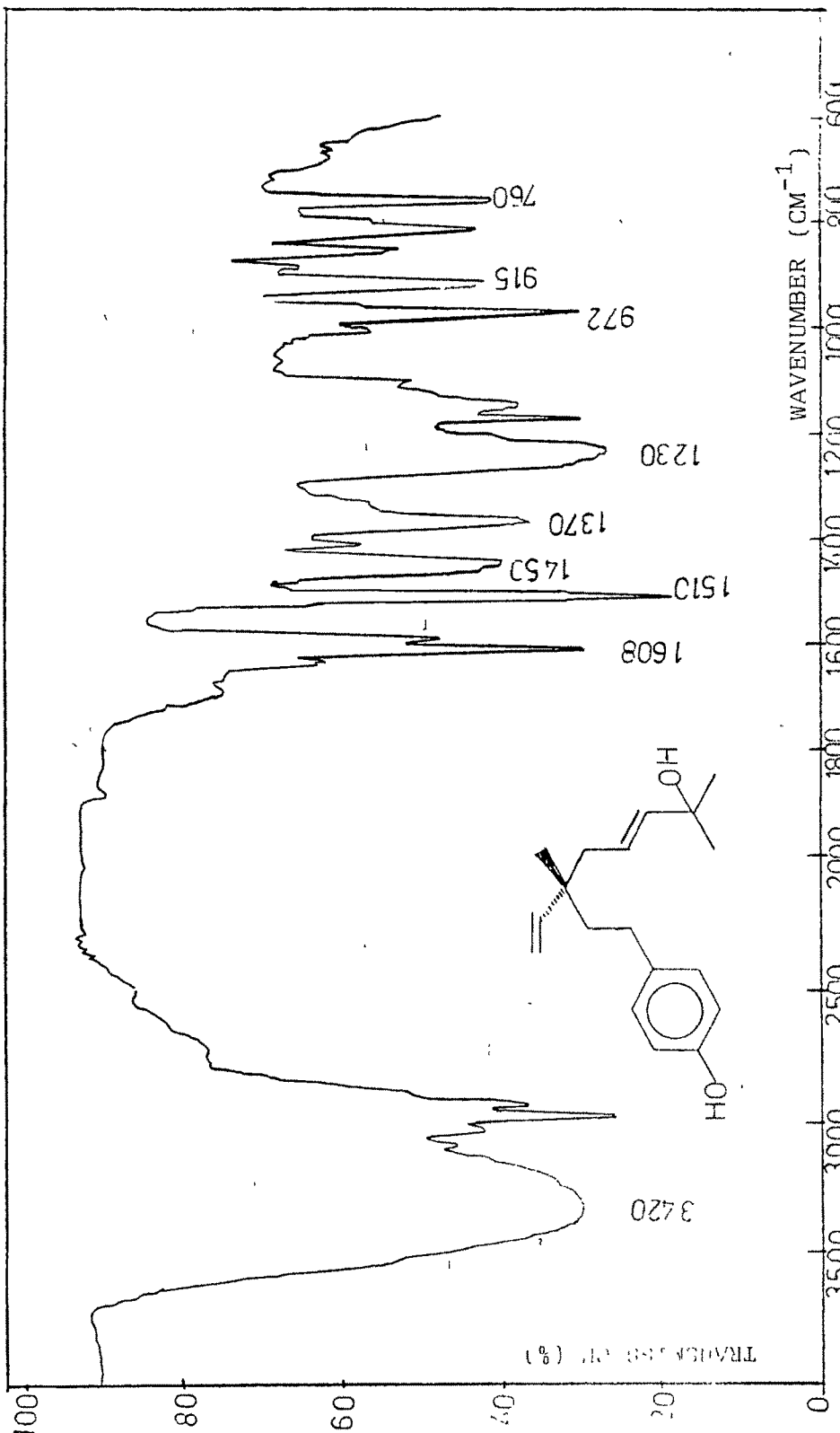


FIG. 12 - IR SPECTRUM OF Δ^5,7-HYDROXYBAKUCHIOL.

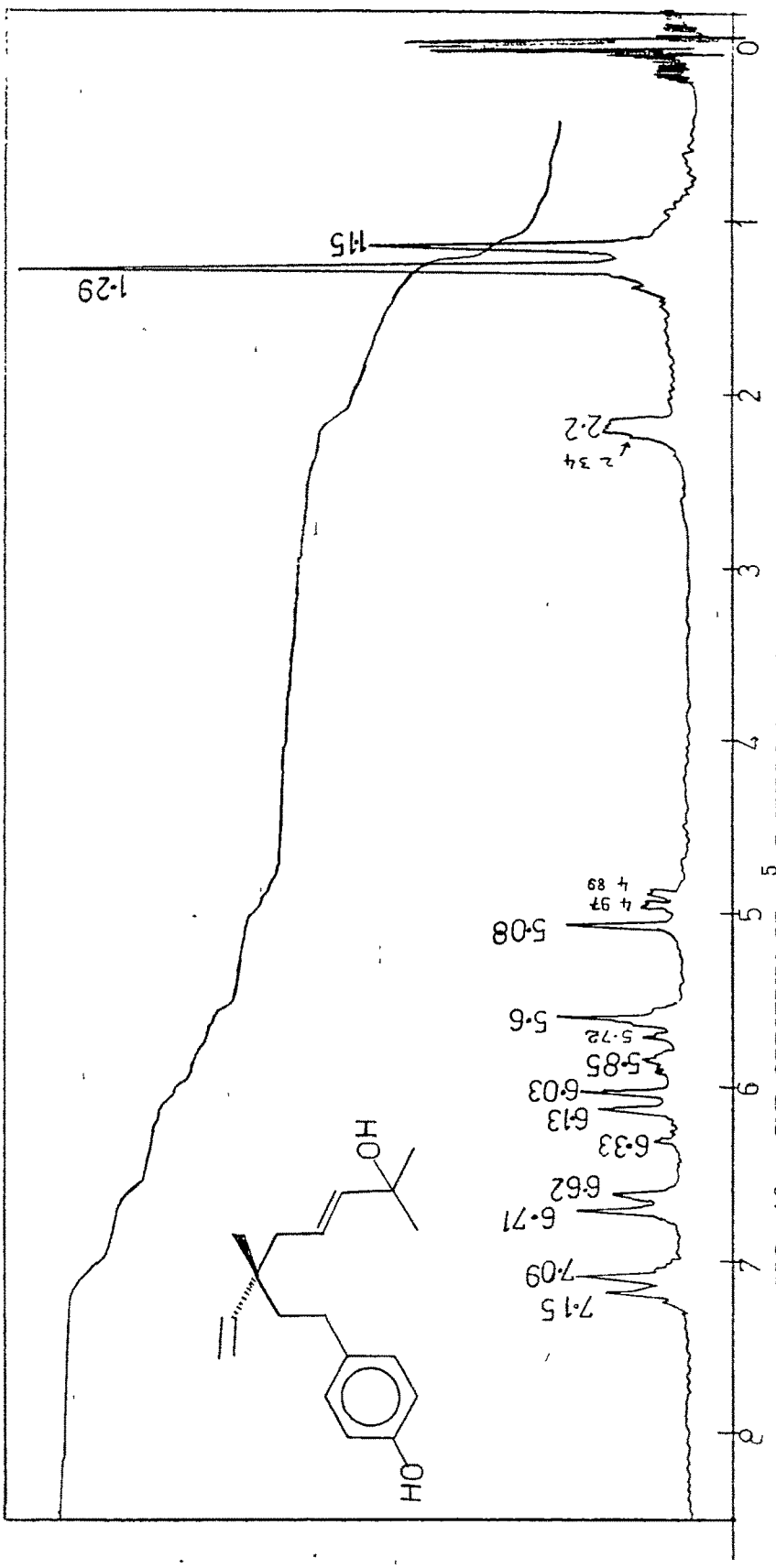


FIG. 13 - PMR SPECTRUM OF Δ^{5},7-HYDROXYBAKUCHIOL.

J_{AB}=9 Hz), $\Delta^{11,12}$(2H, AB-type centred at 6.05 ppm, J_{AB}=18Hz) $\Delta^{1,2}$(3H, m, ABC-type located between 4.7 and 5.93 ppm), $\Delta^{5,6}$(2H,m, located between 5.5 and 6.38 ppm)

^{13}CMR ($CDCl_3$ Fig. 14) see Table-10B.

^{13}C-NMR of bakuchiol and its derivatives

^{13}C-NMR spectorscopy has proved to be an important tool in the structure elucidation of organic compounds. It is used in natural products chemistry in a variety of ways and at various stages of isolation, e.g. checking the identity of two compounds, detection of stereoisomers or other closely related structure. It furnishes key information such as the total carbon number, the number of CH_3, CH_2, CH and quaternary carbons, the number of oxygen and nitrogen containing functions. However, the assignment of signals is sometimes difficult due to overlapping of the signals in complex natural product. ^{13}C NMR of about all types of naturally occuring compounds have been reported.

In the ^{13}C NMR spectrum of bakuchiol and its derivatives [with 'Distortionless Enhancement by Polarization Transfer (DEPT) experiment], the values are assigned as shown in Table-10B. The values are calculated by additivity rule[78] and matching the values with the value given in literature[79] for carbon having identical surroundings. The changes in bakuchiol molecules for the formation of the derivatives namely 6,7-epoxybakuchiol, Δ^{7},6-hydroxy-bakuchiol and Δ^{5},7-hydroxybakuchiol are well confirmed in ^{13}C NMR spectra.

Note : After isolation of 6,7-epoxybakuchiol, Δ^{7},6-hydroxybakuchiol and Δ^{5},7-hydroxybakuchiol in pure form, we found that these compounds were earlier also isolated by V. K. Bhalla (Unpublished work)[76], and were assigned the tentitive structures on the basis of IR, NMR and Mass. We hereby further confirm the structures assigned by ^{13}C NMR.

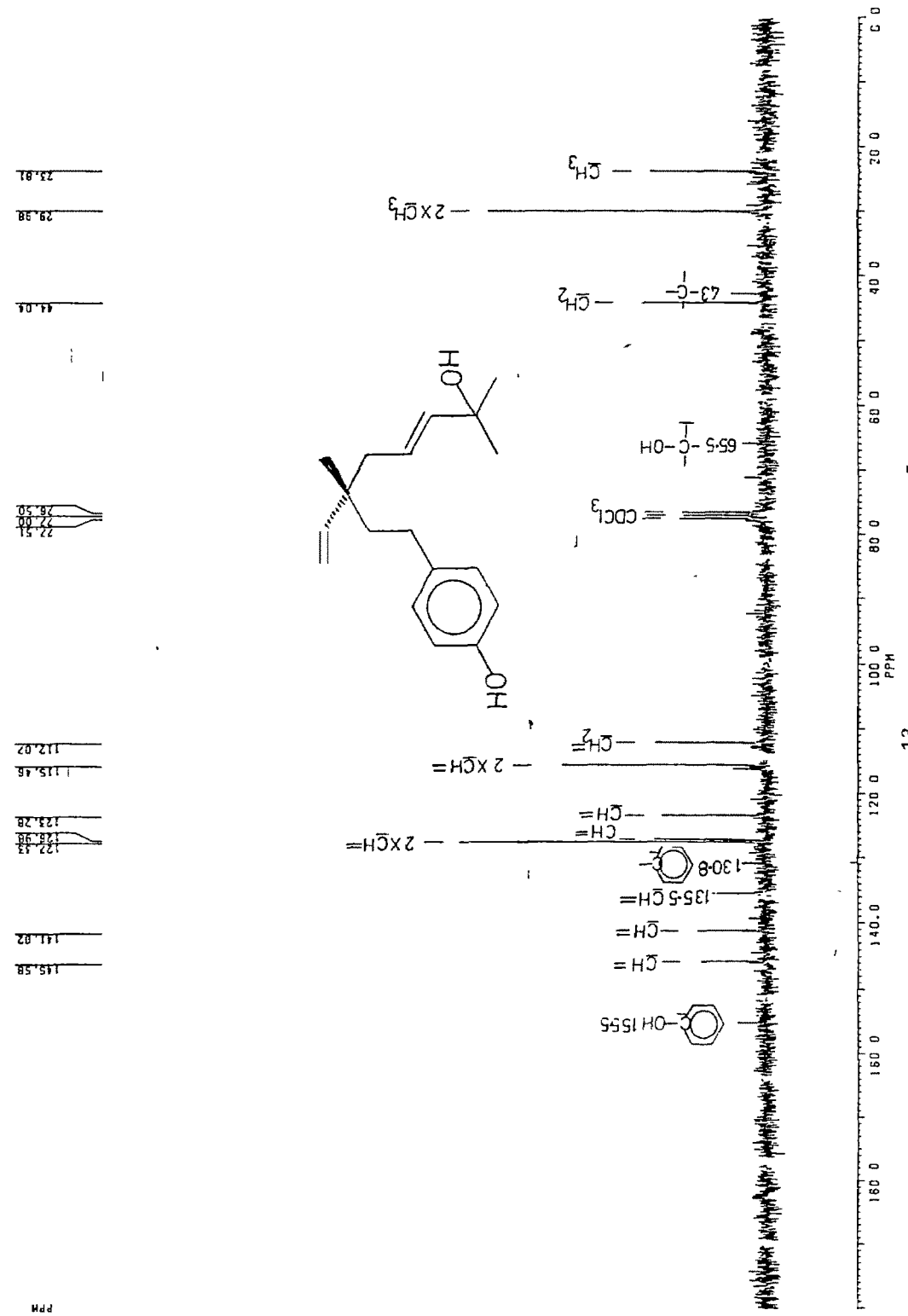


FIG. 14 - ^{13}C NMR SPECTRUM OF Δ^5,7-HYDROXYBAKUCHIOL

Table-10 B : ^{13}C-NMR assignments* of Bakuchiol and its derivatives.

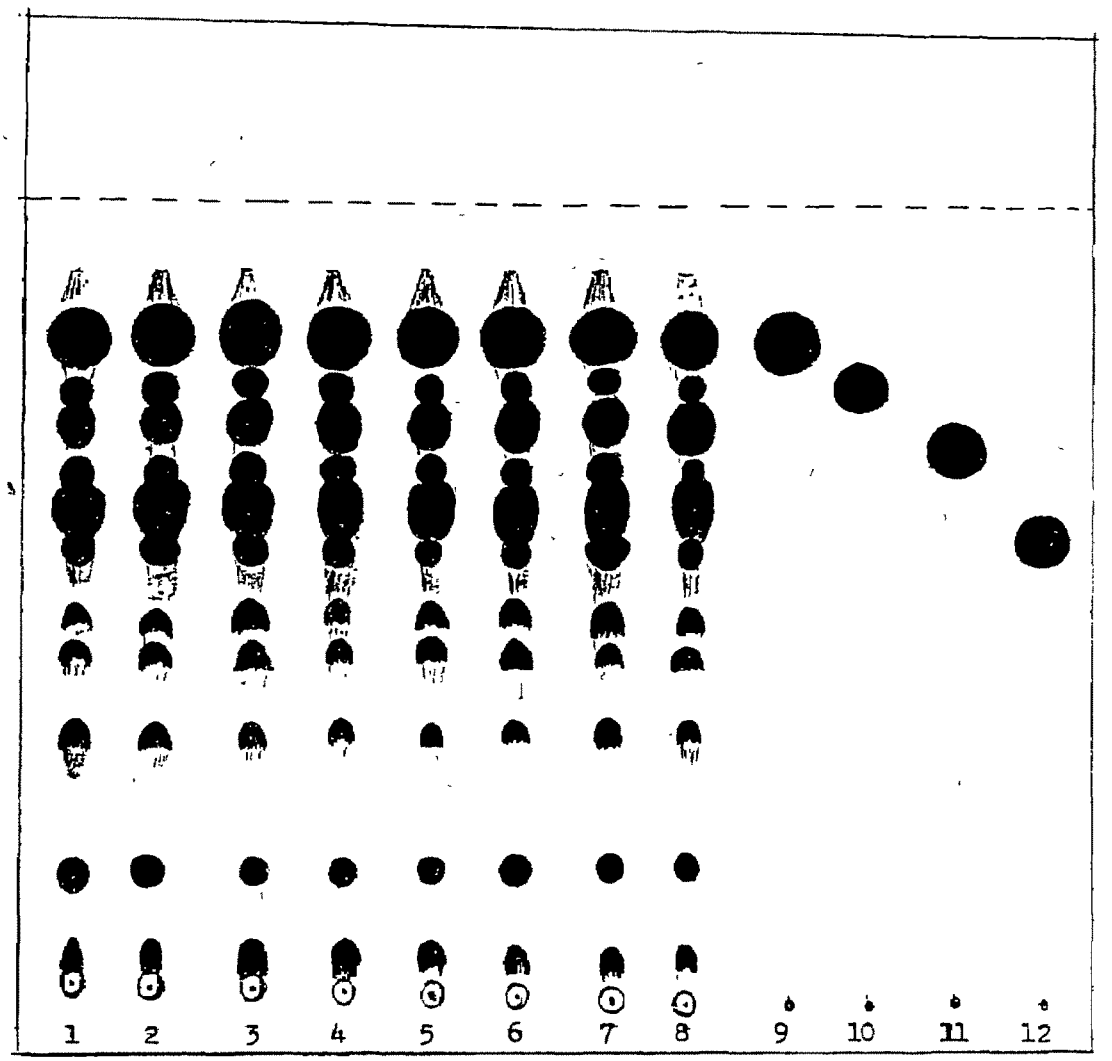
Carbon No.	Bakuchiol	6,7-Epoxy bakuchiol	Δ^{7},6-Hydroxy bakuchiol	Δ^{5},7-Hydroxy bakuchiol.
1	112.1	112.3	112.0	112.1
2	146.0	145.5	145.8	145.6
3	42.6	42.13	42.2	43.0
4	42.6	37.5	37.0	44.0
5	41.4	24.1	29.9	123.3
6	125.1	65.2	76.6	141.0
7	131.3	59.3	147.1	65.5
8	25.82	24.8	111.3	30.0
9	17.69	18.6	17.6	30.0
10	23.4	23.4	23.7	23.8
11	136.2	134.9	135.5	135.5
12	126.6	127.1	126.9	127.0
13	130.5	130.25	130.7	130.8
14	127.6	127.3	127.3	127.3
15	115.7	115.5	115.5	115.5
16	154.37	155.3	155.0	155.5
17	115.7	115.5	115.5	115.5
18	127.6	127.3	127.3	127.3

* ppm from TMS.

Major changes are observed in position of C_5, C_6, C_7 and C_8. In 6,7-epoxybakuchiol the double bond between C_6-C_7 is converted into oxirane ring. In bakuchiol the values for C_6 and C_7 are assigned as 125.1 and 131.3 ppm respectively, which are absent in 6,7-epoxybakuchiol. The assigned values of 65.2 and 59.3 for C_6 and C_7 are well in accord with the values given for trisubstituted epoxy ring[80]. In case of Δ^7,6-hydroxybakuchiol, the double bond between C_6 - C_7 of bakuchiol is shifted to C_7 - C_8 and OH group is substituted at position C_6. The assigned value 76.6 ppm for C_6 is due to the substitution of OH group at C_6 and assigned values for C_7 (131.3 ppm) and C_8 (25.8 ppm) for bakuchiol are shifted to 147.1 and 111.3 ppm respectively. In Δ^5,7-hydroxybakuchiol, double bond between C_6 - C_7 of bakuchiol is shifted to C_5 - C_6 and OH group is substituted at C_7 and the assigned values for C_5 (41.4 ppm) and C_6 (125.1 ppm) for bakuchiol are shifted to 123.3 and 141 respectively due to the double bond between C_5 - C_6.

TMS ether of mixture of above three derivatives of bakuchiol did not separate in GC and showed a single peak (Rt 12.98 mins, Fig. 4), which exactly matched with (Rt~13.8 mins) an extra peak observed during estimation of bakuchiol in both crude and distilled toluene extract. TLC of both distilled and crude toluene extract (Figs. 15,16) of the seeds extracted during different months also showed uneven formation of above mentioned bakuchiol derivatives. Thus it was evident that the variation in the yield of bakuchiol was because of its transformation into its derivatives.

Epoxybakuchiol was found to decompose during distillation and chromatography. In order to know whether or not it gets converted into any of the two hydroxy compounds during distillation or on TLC plate, the compound was synthesized[76] from bakuchiol. The compound showed a single spot on TLC

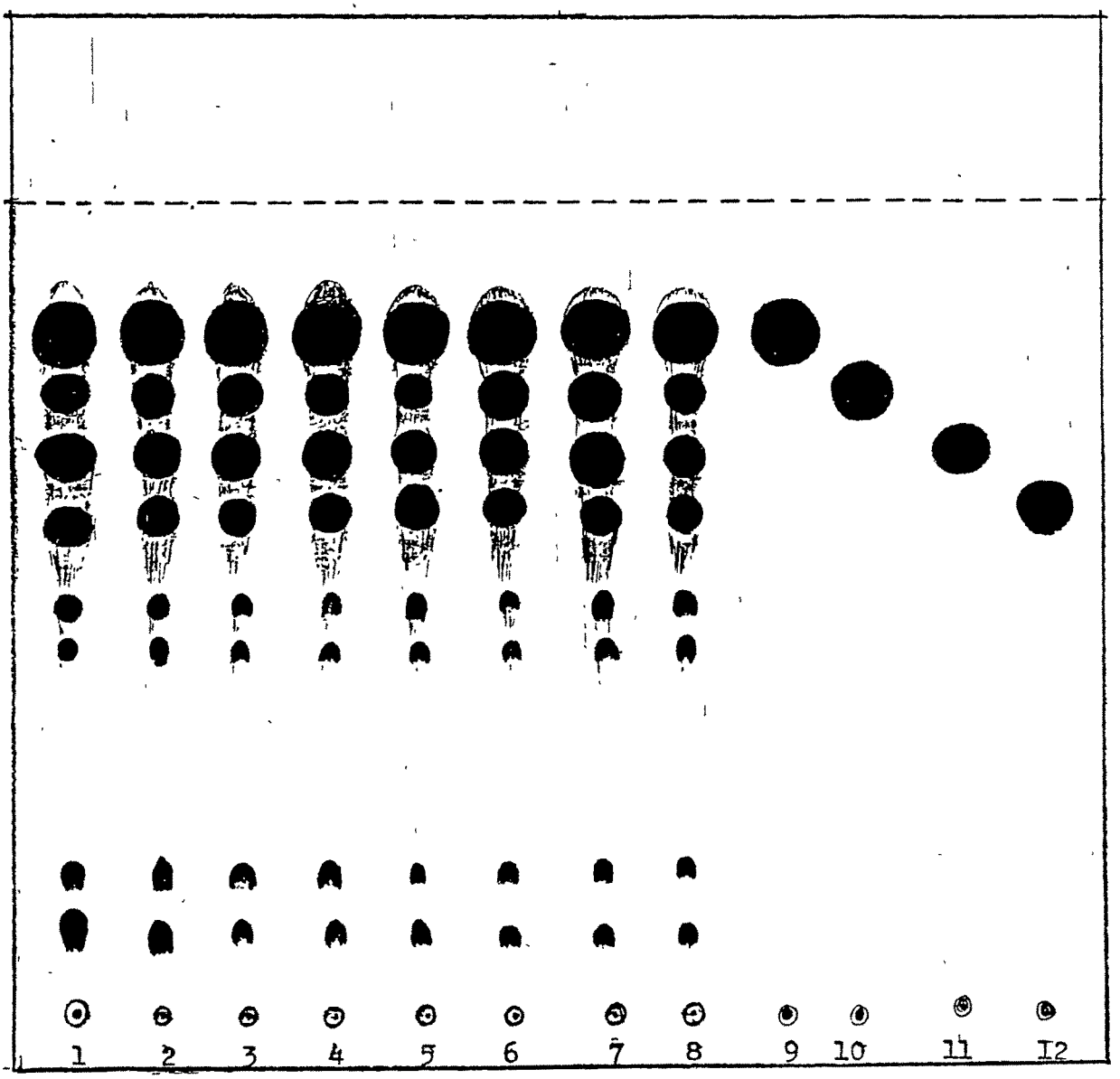


Silica gel - G plate; Solvent system - 20% EtOAc in benzene; Spray reagent - 0.5% anisaldehyde in 2% H_2SO_4 in CH_3COOH.

Seeds were extracted in the month of (1) January (2) February (3) March (4) April (5) May (6) June (7) July (8) December.

Authentic sample of (9) Bakuchiol (10) 6,7-Epoxybakuchiol (11) Δ^{7},6-Hydroxybakuchiol (12) Δ^{5},7-Hydroxybakuchiol.

Fig. 15 : TLC of crude toluene extract of P. corylifolia Linn seeds, extracted in the different months.



Silica gel - G plate; Solvent system - 20% EtOAc in benzene; Spray reagent- 0.5% anisaldehyde in 2% H_2SO_4 in CH_3COOH.

Seeds were extracted in the month of (1) January (2) February (3) March (4) April (5) May (6) June (7) July (8) December.

Authentic sample of (9) Bakuchiol (10) 6,7-Epoxybakuchiol (11) Δ^{7},6-Hydroxy-bakuchiol (12) Δ^{5},7-Hydroxybakuchiol.

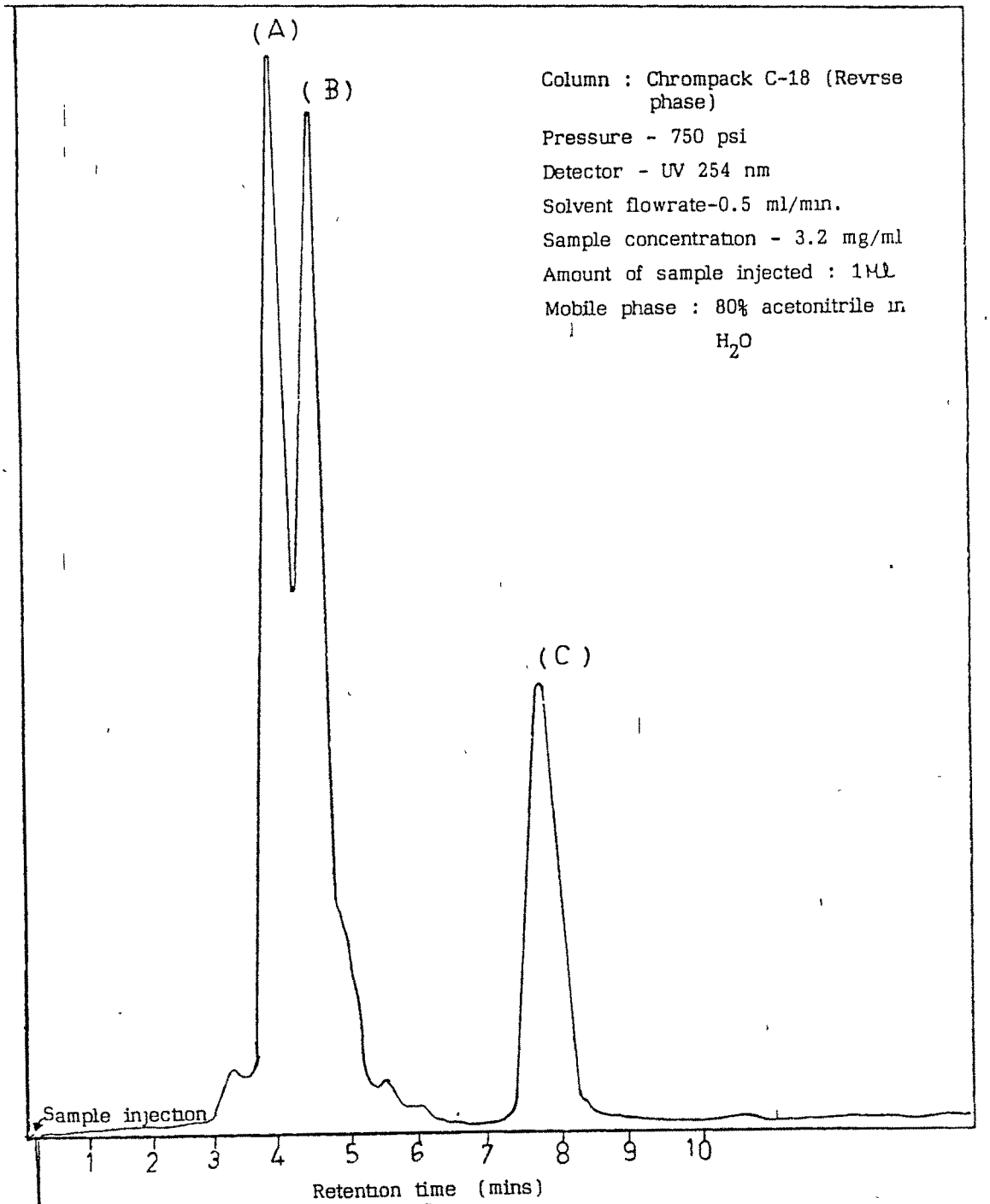
Fig. 16 - TLC of distilled toluene extract of P. corylifolia Linn seeds, extracted in different months.

(Fig. 3). However, when distilled at 170-200°(bath)/0.5 mm, TLC of the distillate showed four spots, but Rf of none of them matched with the Rf of either Δ^{7},6-hydroxybakuchiol or Δ^{5}-7-hydroxybakuchiol. Thus it eliminated the possibility of formation of the hydroxy compounds from the expoxybakuchiol by thermal decomposition or on the SiO_2 gel (TLC or chromatotron).

HPLC of bakuchiol and its derivatives

As the above mentioned bakuchiol derivatives in the extract did not separate in GC, the extract was subjected to HPLC. For HPLC study mixture of accurately weighed bakuchiol, 6,7-epoxybakuchiol, Δ^{7},6-hydroxybakuchiol and Δ^{5},7-hydroxybakuchiol was prepared and dissolved in acetonitrile. The HPLC of the mixture using acetonitrile in water (80:20) as a mobile phase, exhibited only three peaks (Fig. 17), which corresponded to bakuchiol (Rt 7.41 mins), 6,7-epoxybakuchiol (Rt 4.48 mins) and mixture of Δ^{7},6-hydroxybakuchiol and Δ^{5},7-hydroxybakuchiol (single peak, Rt 3.77 mins). Peaks were confirmed by co-injection with pure authentic samples. The correction factor was calculated from the ratio of the observed percentage vs. the actual percentage of the each compound in the mixture. This correction factor was used for the calculation of exact percentage of the compound in an unknown mixture (Table-11).

For HPLC analysis, the crude toluene extracts of seeds extracted in the month of February (contained 3.05% bakuchiol), July (contained 1.9% bakuchiol) and December (contained 2.49% bakuchiol) were weighed accurately and separately purified on chromatotron using 50% ethylacetate in pet.ether as eluent to remove polar impurities. The purified extracts were weighed again and subjected for HPLC. Table-12 represents percentage of each compound in the mixtures.



(A) Δ^{7},6-Hydroxybakuchiol and Δ^{5},7-Hydroxybakuchiol (Rt 3.77 mins)
(B) 6,7-Epoxybakuchiol (Rt 4.48 mins) (C) Bakuchiol (Rt 7.4 mins).

Fig. 17 - HPLC of bakuchiol and its derivatives.

Table-11 : HPLC of bakuchiol and its derivatives (known mixture).

Compound	Wt.of the compound (mg) [Retention time (mins)]	Observed % in the mixture.	Actual % in the mixture	Correction factor.
Bakuchiol	3.2 (7.41)	61.87	46.37	0.7494
6,7-Epoxybakuchiol	2.0 (4.48)	20.99	28.98	1.38
Δ^{7},6-Hydroxybakuchiol	1.11 (3.77)	16.98	15.94 + 8.69 = 24.63	1.45
Δ^{5},7-Hydroxybakuchiol	0.52 (3.77)			

Table-12 : Estimation of bakuchiol and its derivatives by HPLC in crude extracts of P. corylifolia Linn seeds.

Compound	Seeds extracted (month)					
	February		July		December	
	1 (2)	3 (2)	1 (2)	3 (2)	1 (2)	3 (2)
Bakuchiol	46.6 (51.0)	2.77 (3.0)	26.5 (29.0)	1.75 (1.9)	53.85 (53.27)	2.53 (2.49)
6,7-Epoxybakuchiol	11.74	0.694	21.05	1.39	10.98	0.513
Δ^{7},6-Hydroxybakuchiol Δ^{5},7-Hydroxybakuchiol	20.92	1.24	39.22	2.58	20.02	0.938

1- % in crude extract; 2- % estimated by GC; 3-% in seed.

The following conclusions were drawn from the HPLC analysis.

(1) Percentage of bakuchiol in the extract and in the seed, obtained by both HPLC and GC are very close.

(2) Presence of 6,7-epoxybakuchiol, Δ^{7},6-hydroxybakuchiol and Δ^{5},7-hydroxybakuchiol in all the three crude toluene extracts of seeds, prove that all these compounds are originally present in the crude toluene extract and are not derived from bakuchiol during distillation of the extract.

(3) The variation in the bakuchiol content is due to the formation of the expoxy and the hydroxy derivatives.

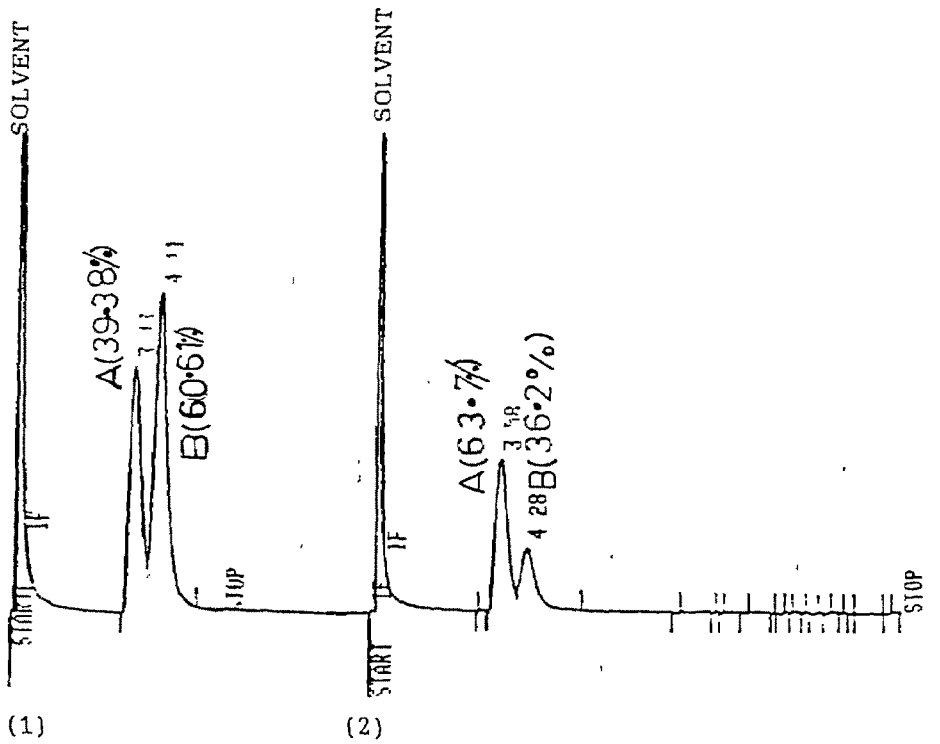
PART - B PSORALEN AND ISOPSORALEN FROM BAKUCHIOL EXTRACTED SEEDS.

The seeds of Psoralea corylifolia Linn, from which bakuchiol was completely extracted out, were utilized for the extraction of psoralen and isopsoralen. Various methods are reported for the isolation of these compounds.[11,23,75,77] After trying several methods, we followed the method reported by Dhar et al.,[75]. In the process, the bakuchiol extracted seeds were pulverized coarsely and the powder was soaked in water for 7 days at room temperature. The seeds were then filtered and completely dried in shade for three days. The dry powder was extracted with hot pet.ether in soxhlet for 8 hrs. The pet.ether extract was concentrated and chilled at 3-5° for 3 hrs. Mixture of psoralen and isopsoralen separated as a white powder, was filtered off and the mother liquor was further concentrated and cooled. Three more crops of the mixture were collected in the same manner (total yield 1.01%). Number of experiments for extraction of psoralen and isopsoralen, were carried out. Results are reported in Table-13. The mixture of psoralen and isopsoralen was obtained as amorphous solid and

was found to be contaminated with other impurities (TLC, solvent system 25% EtOAc in benzene). The mixture (146.02 g) was dissolved in hot methanol (1.5 L), filtered and allowed to stand at 30° for 6 hrs. White needles, which crystallized, were filtered and washed with cold methanol and dried to get 33.88 g of compound, which on TLC showed a mixture of psoralen (Rf 0.58) and isopsoralen (Rf 0.62) freed from impurities. The mother liquor was concentrated and allowed to stand at room temperature. The second crop was collected after 6 hrs. Total six crops of pure mixture of psoralen and isopsoralen (124.8 g, 0.9%) were collected. The ratio of psoralen : isopsoralen[23] was 60.6 : 39.4 in the 1st crop and 36.2 : 63.7 in the mixture of remaining crops (Fig.18).

TABLE - 13 Extraction of psoralen and isopsoralen

Expt. No.	Wt. of the Seeds (kg)	Yield of psoralen-isopsoralen mixture (g)(%)		
		Crude (%)		Crystallised (%)
1.	0.14	1.42	(1.01)	
2.	1.42	13.6	(0.95)	124.85 (0.945)
3.	4.17	48.5	(1.16)	
4.	7.48	82.5	(1.1)	
T O T A L :	13.21			



(1) 1st crop of crystallisation.

(2) 2nd - 6th crop of crystallisation.

(A) Isopsoralen (Rt-3.4 mins) (B) Psoralen (Rt 4.11 mins).

Column 10% OV-4, Temp. 190°C, Detector - FID.

Fig. 18 - GC analysis of psoralen and isopsoralen.

EXPERIMENTAL

For general methodology, see experimental of Chapter-1. ^{13}C-NMR (with DEPT experiment) of derivatives of bakuchiol were carried out at the department of chemistry, Harvard university, Cambridge, Massachusetts, U.S.A. and ^{13}C NMR of bakuchiol was carried out on ^{13}C - NMR spectrometer model JEOL FX 900 FT NMR (frequency ^{13}C 22.5 MHz).

HPLC was carried on Dupont model 848, column reverse phase chrompack C-18, microsphere (100mm x 4.6mm), particle size 3 micron, Condition : Pressure 750 psi, solvent flow rate 0.5 ml/mins, Detector UV (at 254 nm), chart speed 0.5 inch/min, sample concn. 3.2 mg/ml. Amount of sample injected 1 µl, mobile phase 80% acetonitrile in water. Gas chromatography analyses were normally carried out on 10% OV-4 column (stated otherwise). All TLC were run on SiO_2 gel and solvent system normally used was 20% ethylacetate in benzene (stated otherwise). Visualization of the spots was done by spraying with 0.5% anisaldehyde in 2% H_2SO_4 in CH_3COOH or by I_2.

Part - A : Section - 1

Cold extraction of Psoralen corylifolia Linn. seeds with solvent ether.

Whole seeds of P. corylifolia Linn (200 g) were taken in a separating funnel and percolated (extracted) with solvent ether (400 ml) for 24 hrs. Solvent was then drained and replaced by the fresh solvent (350 ml) and percolated for 24 hrs. The same process was repeated again (till the extract showed very faint spot of bakuchiol on TLC). All the three solvent ether extracts were combined and solvent was stripped off. Last traces of the

solvent were removed at 80°/100 mm to get dark yellow viscous gum (24.94 g), which was subjected to chromatography.

TABLE - 14 CHROMATOGRAM

Material	22.79 g adsorbed on 30 g SiO_2 gel.
Adsorbent	SiO_2 gel/IIb, 400 g.
Column dimension	72 cm x 3.5 cm

Fr. No.	Eluent	Vol. of Fraction	Wt. of Fraction (g)	Remarks
1.	Pet.ether	100ml x 3	0.0031	
2.	Pet.ether-benzene (50:50)	1000ml x 2	0.2043	
3.	Pet.ether-benzene(40:60)	250ml x 1	0.7051	
4.	Pet.ether-benzene(40:60)	250ml x 3	4.7853	Almost single spot corresponding to bakuchiol.
5.	Methanol	1000ml x 2	15.7393	Mixture.
		T O T A L :	21.4371	(94.07%)

Fraction 4 was distilled at 160-165/0.9 mm to get 4.6903 g of bakuchiol as yellow liquid (TLC single spot).

Hot extraction of Psoralea corylifolia Linn seeds.

Whole seeds of P. corylifolia Linn (100 g) were placed in a soxhlet and continuously extracted with hot toluene (350 ml) for 24 hrs. The toluene was then distilled off at 80-85°/100 mm and last traces of solvent were removed 80°/20 mm to get dark yellow viscous gum (11.6917 gm). It was subjected to column chromatography.

TABLE - 15 CHROMATOGRAM

Material	11.69 g adsorbed on 12 g silica gel.
Adsorbent	SiO_2 gel/IIb, 200 g
Column dimension	65 cm x 2.8 cm

Fr. No.	Eluent	Vol. of Fraction	Wt. of Fraction (g)	Remarks
1.	Pet.ether	500ml x 4	0.3024	
2.	Pet.ether-benzene(50:50)	200ml x 6	2.0815	
3.	Pet.ether-benzene(40:60)	200ml x 4	2.7038	Almost single spot on TLC corresponding to bakuchiol.
4.	Methanol	1000ml	5.8137	Mixture
		TOTAL:	10.9015(93.25%)	

Fraction 3 was distilled at 160-65°/0.9mm to get 2.6 g of bakuchiol as yellow liquid (TLC single spot).

Methods tried for purification of bakuchiol

(1) Washing of toluene extract with 20%(aq.) methanol.

Solvent free toluene extract (0.47 g) was dissolved in benzene (5 ml) and extracted with aqueous methanol (20% in water) (5mlx3). Benzene was removed at reduced pressure to get benzene soluble portion (0.37 g). Aqueous methanol was removed from the methanol (aq.) soluble portion to get the soluble fraction (0.106 g). Both the portion were then spotted on TLC.

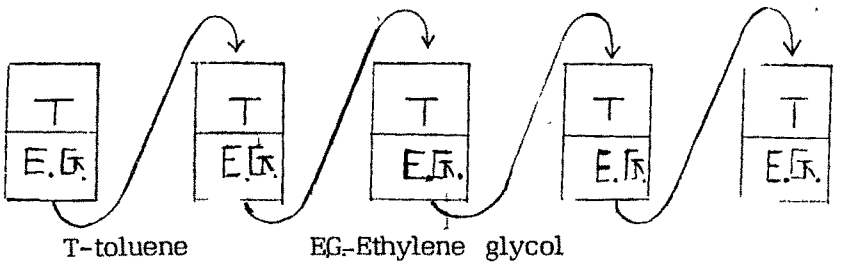
(2) Use of KOH impreganted SiO_2 gel.

SiO_2 gel (100-200 mesh, 100 g) was placed in a 1 litre round bottom flask and to that aq. KOH (20 g KOH in 50 ml H_2O) was added and the slurry thus obtained was rotated on rotavapor for 1 hr for impregnation. Water was then removed under reduced pressure in such a way that there was an increase in the wt. of silica gel by 18 g. Net wt. of KOH impreganted SiO_2 gel was 138 g.

Solvent free toluene extract (2.09 g) was dissolved in toluene (100 ml) in a 250 ml round bottom flask. The above KOH impregnated silica gel (1 g) was added to that and stirred for 5-10 minutes and then SiO_2 gel was filtered and toluene was removed from the filtrate at 80°/100 mm on rotavapor to get the unadsorbed material (1.7965 g). TLC of the filtrate was checked but no change was observed. The same treatment was repeated and total 10 g of KOH impregnated SiO_2 gel was added. Total 1.3286 g material remained unadsorbed after addition of 10 gm KOH impregnated SiO_2 gel.

Counter current distribution

(A) Counter current distribution between toluene and ethylene glycol.



T-toluene EG-Ethylene glycol

Toluene extract (8.07 g) was dissolved in toluene (40 ml) and vigorously shaken with ethylene glycol (20 ml) in the separating funnel No.1. The lower layer of ethylene glycol was separated and transferred to separatory funnel No.2 containing toluene (40 ml) and vigorously shaken. The lower layer was separated and transferred to the 3rd, 4th, and 5th separating funnel subsequently. Thus the toluene extract was distributed between toluene (40mlx5) and the ethylene glycol (20mlx5). All the extracts were checked on TLC. The toluene layer of 1st and 2nd separatory funnels which showed (TLC) least amount of polar impurities, were combined and solvent was removed at 80°/100 mm on rotavapor to get 5.27 g of the residue. The residue was redissolved in toluene (40 ml) and the above procedure was repeated. Again TLC of the toluene layer of 1st and 2nd

separatory funnel showed much pure bakuchiol. Both the layers were combined and solvent removed to get 4.08 g of residue, which was distilled 160-165°/0.9 mm to give 2.0465 g of distillate (25.3 %).

(B) Counter current distribution between toluene and aq. propylene glycol.

Toluene extract (7.44 g) was dissolved in toluene (40 ml) and extracted with propylene glycol-water (2:1, 20 ml). The aq. propylene glycol layer was separated and extracted by counter current method as described above. After checking the TLC, toluene layer of separatory funnel No. 1-3 were combined and solvent removed to get 6.41 g of residue.

(C) Counter current distribution between toluene and alkaline ethylene glycol.

KOH (2g) was dissolved in water (1.1 ml) and diluted to 100 ml with ethylene glycol. Toluene extract (7.55 g) was dissolved in toluene (40 ml) and extracted with alkaline ethylene glycol (20 ml) by the counter current method as above.

Trituration of toluene extract with pet.ether

Toluene extract (95 g) was cooled in an ice bath (3-5°C) and to the viscous gummy material, was added chilled pet.ether (~3°, 100 ml) and stirred manually with glass rod for 10-15 mins and then allowed to settle for 10 mins. The pet.ether was decanted and fresh pet.ether was added (100 ml) and the process was repeated. Thus the extract was repeatedly triturated by chilled pet.ether till the pet.ether layer showed only a faint spot of bakuchiol (100 ml x 15). Distillation of solvent from combined pet.ether extract furnished dark yellow residue (51.43 g). Further purification of bakuchiol was done by following methods.

(1) Counter current distribution of pet.ether soluble portion between ethylene glycol and toluene.

Pet.ether soluble portion (1 g) was dissolved in toluene (10 ml) and extracted with ethylene glycol (5mlx5). Each of the ethylene glycol extract was reextracted with toluene (5mlx4) by counter current method described earlier. Toluene extract of 1st and 2nd separatory funnels containing bakuchiol (TLC) were combined and solvent was removed at 80°/100 mm to give crude extract, which was distilled at 160-165°/0.9 mm to get bakuchiol (0.46 g, TLC pure).

(2) Co-distillation with ethylene glycol.

Pet.ether soluble portion (6.7 g) was placed in a 100 ml round bottom flask and to it ethylene glycol (35 ml) was added. The mixture was subjected to distillation at 130-135°/10 mm. The distillate was transferred to a separatory funnel and extracted with toluene (10mlx5). Combined toluene extracts after usual work-up afforded impure bakuchiol (0.3039 g).

(3) Filtration through alumina.

[3.1] Alumina grade IIIa

Pet.ether soluble portion (51.42 g) was dissolved in pet.ether (500 ml) and neutral alumina (IIIa, 100 g) was added. The slurry was rotated on rotavapor and pet.ether was removed at 50°/100 mm. The adsorbed material was filled in a column and eluted with pet.ether followed by toluene.

TABLE - 16 CHROMATOGRAM

Material	51.42 g adsorbed on neutral alumina. grade-3A(100 gm)
Adsorbent	Alumina/IIIa, 250 g
Column dimension	38 cm x 3 cm

Fr. No.	Eluent	Vol. of Fraction	Wt. of Fraction (g)	Remarks
1.	Pet.ether	500 ml	14.75	
2.	Pet.ether	1000 ml	2.31	
3.	Pet.ether	3000 ml	2.80	
4.	Toluene	2000 ml	5.49	
5.	Toluene	3000 ml	33.34	
6.	Toluene	2000 ml	2.01	TLC showed very faint spot of bakuchiol
		Total :	30.70	

Fraction 1-6 (30.7 g) was distilled at 145-147°/0.7 mm to get yellow viscous liquid (22.2869 g).

[3.2] Alumina grade-IIb

Pet.ether soluble portion (43.2 g) was dissolved in pet.ether (1 lit) and to it alumina (IIb, 302 g) was added. The slurry was rotated on rotavapor and pet.ether was removed at 50°/100 mm. The adsorbed material was filled in a thimble and placed in the soxhlet apparatus and extracted with hot toluene (1 lit) for 15 hrs. Removal of toluene under vacuum at 80° yielded crude bakuchiol (24.9 g), which was distilled at 160-165°/0.9 mm to afford light yellow distillate (18.67 g). Redistillation of the distillate using a small vigreaux column yielded pure bakuchiol (16 g, ~ 95% pure).

FINAL PROCEDURE

The whole seeds of Psoralea corylifolia Linn (10 kg) were percolated with toluene (10 lit) in a 20 l glass aspiratory bottle for 24 hrs. Toluene was drained and replaced by fresh toluene (10 lits) and the same process was repeated twice. All the three extracts were combined and toluene was

stripped off at 80°/10 mm to yield yellow viscous gum (708.6 g). It was cooled in an ice bath (3-5°) and triturated with cold pet.ether (~3°, 250 ml) with mannual stirring for 30 min. and then allowing to stand at 3-5° for 30 mins. Pet.ether layer was decanted and fresh pet.ether was added (250ml). The process was repeated till the pet.ether layer showed only a faint spot of bakuchiol (250 mlx17). All the pet.ether extracts were combined and solvent was removed on rotavapor (50°/100 mm) to get pet.ether soluble portion (350 g) as dark brown liquid. It was dissolved in pet.ether (5 lit) and adsorbed on neutral alumina (IIb, mesh 100-200, 2.45 kg). The slurry was rotated on rotavapor at 40° for 1 hr and then 50°/100 mm to remove pet.ether. The free flowing powder was then filled in a thimble and extracted with toluene (5 lit) in the soxhlet till the extracted solvent showed absence of bakuchiol (15 hrs). Toluene was removed from the extract at 80°/100 mm on rotavapor to yield crude bakuchiol (212.4 g), which was distilled at 145-147°/0.7 mm. Forerum (4g) containing less polar impurity was discarded and TLC pure bakuchiol (160.25 g) was collected.

Part - A **Section - 2**

Estimation of Psoralea corylifolia Linn seeds.

Whole seeds of P. corylifolia Linn were filled in a separatory funnel and percolated with toluene (100 ml) for 24 hrs. Toluene was drained and replaced by fresh toluene (100 ml). The process was repeated for total five times. Toluene was removed at 80°/100 mm on rotavapor from combined toluene extracts to afford dark red viscous liquid (7.96 g). Which was used for the followings.

(1) Estimation of bakuchiol by column chromatography :

1 g of the above extract was chromatographed on SiO_2 gel.

TABLE - 17 CHROMATOGRAM

Material	1 g adsorbed on SiO_2 gel (IIb, 1.2 g)
Adsorbent	20 g SiO_2 gel/IIb
Column dimension	10 cm x 0.3 cm

Fr. No.	Eluent	Vol. of Fraction	Wt. of Fraction (g)	Remarks
1.	Pet.ether	200ml x 1	0.0250	
2.	Pet.ether-benzene(1:1)	200ml x 3	0.3178	Pure bakuchiol(TLC)
3.	MeOH	100ml x 1	0.6001	Mixture
		T O T A L :	0.9420	

Fraction-2 contained TLC pure bakuchiol.

Percentage of bakuchiol in the extract 31.78%

Percentage of bakuchiol in the seeds 2.53%

(2) Gas chromatography

Crude toluene extract (1 g) was distilled at 150-170° at 0.5 mm to yield light yellow oil (0.5123 g). Distillate (50 mg) was dissolved in dry pyridine (5 ml) and to it was added hexamethyldisilazane (HMDS, 0.5 ml) and trimethylsilylchloride (TMCS, 0.5 ml) and the mixture was shaken for 5 mins. Pyridine was distilled off at 90-95°/100 mm. The white solid which was left as a residue, was washed with dry pet.ether (10 ml) and then filtered through filter paper. Removal of pet.ether from filtrate afforded clean liquid, which was subjected to GC analysis (10% OV-4, 190°). Bakuchiol percentage was 82.8% .

percentage of bakuchiol in the extract =

$$\frac{0.5123 \times 82.8}{100} = 42.41\ \%$$

Percentage of bakuchiol in the seed =

$$0.4241 \times 7.96 = \frac{3.37}{100} \times 100 = 3.37\ \%$$

(3) GC analysis using cetyl alcohol as internal standard (IS).

Cetyl alcohol (GC purity 97.36%) was weighed accurately in a small pear shaped flask (0.0971 g). In the same flask was weighed bakuchiol (99.31%, 0.1987 g). Dry pyridine (10 ml) was added and both the compounds dissolved by shaking. TMCS (2 ml) and HMDS (2 ml) were added and stirred at room temp. for 5 mins. and then worked-up as usual. Product was analysed by GC.

$$\text{Area correction factor (A.C.F.)} = \frac{\text{Wt. of bakuchiol x Area of IS x Purity of IS}}{\text{Wt. of IS x Area of bakuchiol x Purity of bakuchiol}}$$

TABLE - 18 Calculation of Area Correction Factor

No.	Wt. of bakuchiol (99.31%)g	Wt. of IS (97.36%)g	Area of IS	Area of bakuchiol	A.C.F.	Mean A.C.F.
1.	0.1987	0.0971	32.659	66.055	0.994	
2.	0.1196	0.0917	42.98	55.904	0.985	
3.	0.0999	0.1116	52.784	45.885	1.009	1.00125
4.	0.1109	0.0915	46.192	52.497	1.095	

Sample Analysis

Accurately weighed cetyl alcohol ($\sim$ 50 mg) was mixed with equal amount of (accurately weighed) the distilled toluene extract and silylated with HMDS (1 ml) and TMCS (1 ml) in dry pyridine as usual. The residue after usual work-up was analysed by GC and actual purity of bakuchiol was found out by the formula :

$$\% \text{ purity} = \frac{\text{A.C.F. x Wt. of I.S. x Area of bakuchiol x 100}}{\text{Wt. of sample x Area of I.S.}}$$

TABLE - 19

No.	Wt. of i.s. (g)	Area of i.s.	Wt. of sample (g)	Area of bakuchiol	% of purity	Mean purity
1.	0.0645	51.21	0.0727	38.68	65.28	65.65
2.	0.0361	39.43	0.064	46.45	66.013	

Percentage of bakuchiol in the seeds = $\frac{65.65 \times 4.08}{100}$ = 2.676 %

Percentage of bakuchiol in the extract = $\frac{51.23 \times 65.65}{100}$ = 33.63 %

Section - 3 . Maturity of seeds and bakuchiol content

General procedure for estimation of bakuchiol in seeds.

Seeds of Psoralea corylifolia Linn were cultivated in our nursery. Matured seeds were collected in beginning of January and stored in a porous cotton bag at room temperature. For the estimation of bakuchiol, seeds were used from the stored seeds. The seeds extracted in the month of January to July and December were estimated. The procedure for analysis was as follows.

100 g of just matured black seeds were taken out and moisture content was found out by heating 10 g of the seeds at 120° for 8 hrs. The seeds were cooled in a desiccator to 30° and reweighed. The moisture content was 10.57%. The seeds were then placed in a separatory funnel and extracted with toluene (200 ml) at room temp. for 24 hrs (5 times). Toluene was distilled off from the extract at 90°/100 mm to get the residue. Last traces of solvent were removed at 60°/5 mm to get 3.76 g of toluene extract, which was flushed with nitrogen and stored in freeze at 10-12°. In December 0.8184 g of the extract was distilled at 150-250°(bath)/0.5 mm to get 0.5301 g of the distillate.

Accurately weighed cetyl alcohol (internal standard) and distillate were mixed and silylated with TMCS (1 ml) and HMDS (1 ml) in dry pyridine (5 ml). The residue after usual work-up was analysed by GC and percentage purity was calculated by the formula :

$$\% \text{ Purity} = \frac{\text{A.C.F. x Wt. of I.S. x Area of Bakuchiol x 100}}{\text{Area of I.S. x Wt. of Sample}}$$

A.C.F.=1.00125

TABLE - 20

No.	Wt. of I.S. (g)	Area of I.S.	Wt. of Sample (g)	Area of bakuchiol	% purity	Mean purity
1.	0.0762	57.447	0.0436	36.382	90.46	90.76
2.	0.0447	61.775	0.0211	32.066	91.06	

Percentage of bakuchiol in seed = $\frac{0.5301 \times 9076 \times 3.76}{0.8184 \times 90\text{g}} = 2.456\ \%$

Toluene extract of February, May and July were also analysed without distillation (crude) in the same way.

Separation of psoralen and isopsoralen from the toluene extract of green seeds.

Immature fresh green seeds of P. corylifolia Linn were collected in December. 147 g of these seeds were extracted with toluene (200 ml x 5) for 24 hrs (each). Solvent was removed from the combined extract to get 3.68 g of the extract. 1.47 g of the extract was distilled at 120-150°(bath)/ 0.5 mm to get 0.8066 g of the distillate. The solidified distillate was diluted with pet.ether (10 ml). The white powder which was separated, was filtered, washed with chilled pet.ether and dried (120 mg). It was found to be a mixture of psoralen and isopsoralen by direct comparison with an authentic sample (mixed TLC, solvent system 20% EtOAc in benzene, spray reagent 5% methanolic NaOH followed by visualisation in UV or I_2 vapour). GC analysis (10% OV-4, 190°, FID) indicated that the ratio of psoralen : isopsoralen

was 87.5 : 12.5 %.

Section - 4 (New constituents)

Isolation of bakuchiol and related compounds.

Distilled toluene extract was subjected to chromatographic separation on chromatotron.

TABLE - 21 CHROMATOGRAM

Material 0.8 g

Adsorbent Silica gel-G (230 mesh) with 17.5 % $CaSO_4$

Thickness of plate (rotor) 2 mm

Fr. No.	Eluent	Vol. of Fraction	Wt. of Fraction (g)	Remarks
1.	Benzene	10ml x 7	0.4736	TLC pure(100%) compound.
2.	Benzene	10ml x 17	0.0950	Colourless viscous liquid 75% TLC pure compound.
3.	Benzene	10ml x 2		
4.	Benzene:EtOAc(95:5)	10ml x 4	0.0854	80% TLC pure comp.
5.	Benzene:EtOAc (95:5)	10ml x 4	0.007	Mixture
6.	Benzene:EtOAc (95:5)	10ml x 8	0.0025	80% TLC pure comp.
7.	Methanol	10ml x 10	0.0438	Mixture

Fraction-1 (0.47 g) : This fraction contained bakuchiol , identified by direct comparison with an authentic sample (mixed GC, TLC, NMR, IR).

Fraction-2 (95 mg) : This fraction contained 75% pure viscous liquid. The compound was identified as 6,7-epoxybakuchiol by direct comparison with synthetic (authentic) compound. (superimposable NMR, IR, mixed GC, TLC, HPLC).

Fraction-4 (85 mg) : This fraction was further purified on chromatotron (SiO_2 gel, 1 mm plate) by eluting with benzene-ethylacetate (98:2) to give

70 mg yellow liquid. Which was distilled at 185-200°(bath)/0.5 mm to furnish 60 mg TLC pure compound. TMS ether of the distillate showed 90% purity. The compound was identified as Δ^{7},6-hydroxybakuchiol by direct comparison of NMR, IR with that of authentic compound.

Fraction-7 (25 mg) : This fraction also subjected to chromatographic purification on chromatotron (SiO_2 gel, 1mm plate) and eluted with benzene-ethylacetate (97:3) to furnish 16 mg TLC pure compound, GC analysis of its TMS ether revealed 89% purity. The compound was identified as Δ^{5},7-hydroxybakuchiol by direct comparison of NMR, IR with that of authentic compound.

Synthesis of 6,7-epoxybakuchiol

Bakuchiol (5.12 g, 0.02 mole) was dissolved in methylene chloride (20 ml) and chilled to 5°. To this solution was added perbenzoic acid in methylene chloride (1 mole equivalent 29 ml, containing 0.0966 g/ml). The mixture was kept in a refrigerator (~8°) for 10 hrs. The reaction was monitored by TLC. After completion of the reaction, it was wahed with saturated solution of Na_2SO_3 (20 ml x 5), H_2O(25 ml x 2), 10% aq. Na_2CO_3 (20 ml x 7), water (25 ml) and finally with brine (25 ml x 2), dried over Na_2SO_4 and solvent removed to get light yellow viscous oil (5.41 g). Which was subjected to purification on chromatotron.

TABLE - 22 CHROMATOGRAM

Material 0.138 g

Adsorbent SiO_2 gel-G (mesh 230) with 17.5% $CaSO_4$

Thickness of plate (rotor) 1 mm.

Fr. No.	Eluent	Vol. of Fraction	Wt. of Fraction (g)	Remarks
1.	Benzene-EtOAc (94:6)	10ml x 1	0.006	
2.	Benzene-EtOAc (94:6)	10ml x 5	0.009	

3.	Benzene-EtOAc (94:6)	10ml x 2	0.080	TLC Single spot.
4.	Benzene-EtOAc (94:6)	10ml x 14	0.011	Mixture
5.	Methanol	10ml x 5	0.021	
		T O T A L :	0.121	

Fraction-3 was identified as 6,7-epoxybakuchiol by direct comparison of spectral data with the authentic compound.

HPLC of Psoralea corylifolia Linn seeds extract

For finding out area correction factor for each peak, a standard mixture consisting of pure bakuchiol, 6,7-epoxybakuchiol, Δ^{7},6-hydroxybakuchiol, Δ^{5},7-hydroxybakuchiol was prepared and analysed by HPLC.

$$\text{Correction factor} = \frac{\text{Actual \% in the mixture}}{\text{Observed \% in HPLC}}$$

where observed % was the wt. of the area covered by the peak on tracing paper.

TABLE - 23

Compound	Wt.of the compound (mg)	Area of the sample (mg) (i.e. Wt. of the tracing paper	Actual %	Observed %	Correction factor.
Bakuchiol	3.2	44.8	46.37	61.87	0.7494
6,7-Epoxybakuchiol	2.0	15.2	28.98	20.99	1.38
Δ^{7},6-Hydroxybakuchiol & Δ^{5},7-Hydroxybakuchiol	1.7	12.3	24.63	16.98	1.45

For general HPLC analysis, the method used was as follows. Toluene extract of the seeds was accurately weighed (~ 100 mg) and dissolved in pet.ether-ethylacetate (1:1, 2 ml). It was loaded on SiO_2 gel plate (1 mm)

of chromatotron and eluted with pet.ether-ethylacetate (1:1, 200 ml). The eluate was filtered through filter paper. Solvent was distilled off to give purified extract, which weighed (~ 90 mg) The purified extract was dissolved in acetonitrile (1 ml) and subjected to HPLC. Residue from the chromatotron plate was eluted with MeOH.

TABLE - 24

Month	Wt. of the crude ext. (mg)	Wt. of the purified ext. (mg)	Compound.*(A,B,C)	Observed %	Corrected % (i.e.Obs. % x C.F.)	% in crude toluene extract	% in seeds
February	104.5	93.8	A	16.08	23.31	20.92	1.24
			B	9.48	13.08	11.74	0.694
			C	69.3	51.93	46.61	2.77
July	69.3	60.2	A	31.14	45.15	39.22	2.58
			B	17.56	24.23	21.05	1.39
			C	40.74	30.53	26.52	1.75
December	179	176	A	14.05	20.37	20.02	0.938
			B	8.08	11.15	10.96	0.513
			C	73.07	54.76	53.85	2.523

*A - Mixture of Δ^{7},6-Hydroxybakuchiol and Δ^{5},7-Hydroxybakuchiol.

B - 6,7-Epoxybakuchiol

C - Bakuchiol.

Part - B **Extraction of psoralen and isopsoralen from bakuchiol extracted seeds.**

(1) Successive extraction of bakuchiol extracted seeds :

Seeds of Psoralea corylifolia Linn. after extraction of bakuchiol were dried and powdered. The powder was filled in the soxhlet and extracted with hot pet.ether (1 lit) for 9 hrs. The same seeds were further extracted with hot ethanol (1 L) for 12 hrs. Distillation of pet.ether from the pet.ether extract yielded 14 g of dark brown oil. Removal of alcohol from alcohol

extract gave 12 g of viscous gummy material.

Pet.ether extract was dissolved in pet.ether (14 ml) and cooled to 8-10°. Psoralen and isopsoralen separated as crystalline powder (7 mg).

(2) Percolation of bakuchiol extracted seeds with toluene-ethylacetate (7:3)

Bakuchiol extracted seeds (48 g) were steeped in toluene-ethylacetate (7:3, 100 ml) in a separatory funnel for 24 hrs. Solvent was then drained and fresh solvent (100 ml) was added and extracted for 24 hrs, after which solvent was drained. The process was repeated thrice. Solvent was removed on water bath from the combined extracts to afford 8 g of dark brown oil. Which failed to crystallise psoralen and isopsoralen in pet.ether.

(3) Extraction of bakuchiol extracted seeds with pet.ether and purification of the extract by neutral alumina.

Bakuchiol extracted seeds (213 g) were placed in a soxhlet and extracted with hot pet.ether (1 lit) for 24 hrs. The extract after usual work up afforded 52 g of dark brown oil. Which was adsorbed on neutral alumina (33 g, IIb) and extracted in the soxhlet with hot pet.ether (1 lit) for 3.5 hrs. The pet.ether extract thus obtained was concentrated to 30 ml and kept at 3-4° for 10 hrs. A white crystalline mixture of psoralen and isopsoralen (105 g) separated (m.p. 130-34°).

(4) Extraction of water extracted powdered seeds with pet.ether.

Bakuchiol extracted seeds (140 g) were pulverized coarsely and soaked in water (500 ml) for 7 days at room temperature. The seeds were then filtered through a cloth and allowed to dry in air under shade at room

temperature for 3 days. The dried seed powder was then extracted with hot pet.ether (1 lit) in the soxhlet for 8 hrs. The pet.ether extract was then concentrated to 30 ml and kept at 3-5° for 3 hrs. The white crystalline powder which separated was filtered and dried. The mother liquor was further concentrated and chilled to get one more crop. Two more crops were obtained in the similar way to yield 1.42 g of the mixture of psoralen and isopsoralen.

REFERENCES

1. B. Mukherji, J. Sci. Industr. Res. 15A, No.5 suppl. 1-12 (1956).

2. K. R. Kirtikar and B.D. Basu, Indian Medicinal plants Vol.-1, Lalit Mohan Basu, Allhabad, 717 (1935).

3. United States Dispensatory (J.B. Lippincott, Philadelphia), 1959 (1947).

4. H.S. Jois and B.L. Manjunath, (A) Ber. dtsch. Chem. Ges., 70B, 434 (1937). (B) Proc. Indian Sci. Congr. 21, 243 (1934); (C) C.A. 30, 4855 (1936).

5. K.K. Chakravarti, A. K. Bose and S. Siddiqui, J. Sci. Industr. Res. 7B, 24 (1948).

6. H. N. Khastgir, P. C. Duttagupta and P. Sengupta, Tetrahedron 14, 275 (1961).

7. G. Mehta, Ph.D. Thesis. University of Poona (1966).

8. G. Mehta, U. R. Nayak and Sukh Dev, Tetrahedron Lett. 38, 4561 (1966).

9. G. Mehta, U. R. Nayak and Sukh Dev, Tetrahedron 29, 1119 (1937).

10. A.S.C.P. Rao, V. K. Bhalla, U. R. Nayak and Sukh Dev, Tetrahedron 29, 1127 (1973).

11. T. R. Seshadri and C. Venkatarao, Proc. Indian Acad. Sci. 5A, 351 (1937).

12. T. R. Seshadri and M. S. Sood, Indian J. Chem. 1, 291 (1963).

13. G. K. Gupta, J. L. Suri, B. K. Gupta and K. L. Dhar, Phytochemistry 21, 2149 (1982).

14. B. K. Gupta, G. K. Gupta, K. L. Dhar and C. K. Atal, Phytochemistry 19(19), 2034 (1980).

15. G. K. Gupta, K. L. Dhar and C. K. Atal, Phytochemistry 16(3), 403 (1977).

16. Z. Guang-Fang et al., Yao-Hsueh. Hsueh. Pao. 14(10), 605 (1979).

17. V. K. Bhalla, U. R. Nayak and Sukh Dev, Tetrahedron Lett. No.20, 240 (1968).

18. B. S. Bajwa, P. L. Khanna and T. R. Seshadri, Curr. Sci., 41, 814 (1972).

19. B. S. Bajwa, P. L. Khanna and T. R. Seshadri, Indian J. Chem., 12, 15 (1974).

20. B. S. Bajwa, P. L. Khanna and T. R. Seshadri, Curr. Sci., 41, 882 (1972).

21. A. C. Jain, P. Lal and T. R. Seshadri, Indian J. Chem., 7, 1072 (1969).

22. A. C. Jain, P. Lal and T. R. Seshadri, Tetrahedron, 26, 2631 (1970).

23. H. N. Khastigir, P. C. Dutta and P. Sengupta, Indian J. Appl. Chem., 22, No. 2 (1959).

24. B. K. Gupta, G. K. Gupta, K. L. Dhar and C. K. Atal, Phytochemistry, 19, 2232 (1980).

25. B. K. Gupta, G. K. Gupta, K. L. Dhar and C. K. Atal, Indian Perfume, 23, 174 (1979).

26. A. C. Jain, G. K. Gupta and P. R. Rao, Indian J. Chem., 12, 659 (1974).

27. S. R. Gupta, T. R. Seshadri and G. R. Sood, Indian J. Chem., 13, 632 (1975).

28. G. K. Gupta, J. L. Suri, K. L. Dhar and C. K. Atal, Phytochemistry, 17, 164 (1978).

29. J. L. Suri, G. K. Gupta, K. L. Dhar and C. K. Atal, Phytochemistry, 17, 2046 (1978).

30. J. L. Suri, G. K. Gupta, K. L. Dhar and C. K. Atal, Phytochemistry, 19, 336 (1980).

31. B. K. Gupta, G. K. Gupta, K. L. Dhar and C. K. Atal, Phytochemistry, 19, 2034 (1980).

32. R. N. Chopra, Indigenous Drug of India (Art press, Calcutta), 367, (1933).

33. K. R. Kirtikar and B. D. Basu, Indian Medicinal Plants I, 717-21 (1935). Edited by E. Blatter and J. F. Caius, Delhi (India).

34. K. L. Dey; Indigenous Drugs of India, Tacker Spink and Company, Calcutta, pp. 258 (1896).

35. G. Panja, Ind. J. Ven. Dis. 13, 56 (1947).

36. S. K. Zutshi, S. K. Joshi and M. M. Bokadia, Indian J. Med. Res., 64, 854 (1976).

37. G. S. Grover and J. T. Rao, Indian Perfume 23, 135 (1979).

38. S. K. Sharma and V. P. Singh, Indian Drugs Pharmaceut Ind. 14(1), 3 (1979).

39. K. C. Gupta, M. C. Bhatia and C. L. Chopra, Bull. Reg. Res. Lab. Jammu 1, 59 (1962).

40. S. K. Zutshi and A. W. Bhagwat, Indian J. Physiol. Phramacol 21, 165 (1977).

41. (A) K. N. Gaind, R. N. Dhar and R. N. Kaul, Ind. J. Phrama., 26, 141 (1964).

(B) K. N. Gaind, R. N. Dhar, B. N. Chopra and R. N. Kaul, Indian J. of Pharm. 27, 198 (1965).

42. K. C. Gupta, C. L. Bhatia, C. L. Chopra and I. C. Chopra, R.R.L. Bulletin, Jammu, 1, 59 (1962).

43. R. H. Singh, R. L. Khosa and B. B. Upadhya, J. Res. Indian Med. 9(2), 65 (1974).

44. K. K. Anand, M. L. Sharma, B. Singh and B. J. Rayghatak, Indian J. Exp. Biol. 16, 1216 (1978).

45. Psoralen and radiant energy, proceedings of Symposium, J. Invest. Dermatology, 32, 131-91 (1959).

46. A. Pakarshi, Annals of Bioche. and Exp. Medicine, 23, 9, 358 (1963).

47. A. Barua, U. K. Banik and D. P. Chakraborti, Am. Biochem. Exp. Med. 21, 139 (1961).

48. K. Vijayalakshmi, S. D. Mishra and S. K. Prasad, Indian J. Entmol. 41, 326 (1979).

49. N. Chandhoke and B. J. Rayghatak, Indian J. Med. Res., 63, 833 (1975).

50. A. S. Bondarenko, B. E. Aizenman, L. A. Bakina and I. S. Kozhina Rastit. Resur. 10(4), 583 (1974).

51. I. S. Kozhina et al., Tr. Sezda Mikrobiol. Ukr. 208-9. Edited by Zatula, D. G. "Naukov Dumka", Kiev, U.S.S.R.

52. A. A. Meshcheryakov et al., Rastit Resur 13(3), 460 (1977).

53. R. Kaul, Arzneim Forsch 26(4), 489 (1976).

54. V. A. Prikhod'Ko and A. S. Bondarenko, Mikrobiol. Zh.(Kiev), 41(4), 400 (1979).

55. V. A. Prikhod'Ko, A. S. Bondarenko and E. L. Mishenkova, Mikrobiol Zh.(Keiv) 45(5), 646 (1980).

56. N. K. Joshi, H. B. Mansukhani and M. S. Chadha, Abstract of paper, All India Insect Chemostriland Research workers conference, Bangalore, Feb. 1975, p. 20.

57. I. H. Rogers, J. F. Manville and T. Sahota, Canad. J. Chem. 52, 1192 (1974).

58. P. Bhan, R. Soman and Sukh Dev, Agric. Blol. Chem. 44(7), 1483 (1980).

59. B. Vig and R. Kanwar J. Indian Chem. Soc., 61, 893 (1984).

60. Private communication with Dr. R. K. Razdan. The John C. Sheehan Institute for Research. Inc. Sharps Associates, Cambridge, Mass. 02138-(617)3542800.

61. B. Mukerji, J. Sci. Ind. Res. 15A(5) Suppl., 1 (1956).

62. B. B. Gokhale, Antiseptic 59, 615 (1962).

63. A. K. Banerjee, A. Rao and N. Basu, Bull Cal Sch Trop Med. 8(2), 66 (1960).

64. R. H. Singh and G. N. Chaturvedi, Indian J. Dermatol Venereol 32, 113 (1966).

65. V. N. Sehgal, V. L. Rege and V. N. Kharangate, Int. J. Dermatol 17, 243 (1978).

66. L. Marquis and G. M. Rangwala, Indian J. Dermatol Venereol Leprol 46, 287 (1980).

67. R. Ali and S. C. Agarwala, J. Sci. Ind. Res. 21C, 321 (1962).

68. R. Ali and S. C. Agarwala, Indian J. Biochem. 2, 271 (1965).

69. R. V. Noronha, Ger. Offen. DE. 3417234 (Cl.A 61,K 31/144) 14th Nov. 1985, Appl. 10th May 1984. C.A. 104 : 24216s.

70. Private communication with Dr. Rudolf V. Noronha, Falk 4a, 5024, Pulheim-1, Germany.

71. L. C. Craig and D. Craig, Technique of Organic chemistry, Second edition, edited by A. Weissberger. Published by Interscience INC. Vol. 3 part one, p. 149 (1956).

72. E. Heftmann, Chromatography, published by Van Nostrand Reinhold Company, New York, p. 695 IInd edition (1966)

73. C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, J. Amer. Chem. Soc. 85, 2495 (1963).

74. D. Harvey and D. E. Chalkley, Fuel 34, 191 (1955).

75. S. Bhattacharji and M. L. Dhar, Indian 59, 265 (1958).

76. V. K. Bhall, Ph.D. Thesis (Poona University) (1968).

77. H. Antonine (France-Indian pharmaceuticals (P) Ltd.). Brit 1,212,134 (Cl. Co7d, A 61 K), 11 Nov. 1970 [CA 74, 50669 y (1971)].

78. P. Clerc and S. Simon, Spectral data for structure determination of organic compounds, published by Springer-Verlag, Berlin Heidelberg, New York (1983).

79. G. C. Levy, R. L. Lichter and G. L. Nelson, Carbon-13 NMR spectroscopy, A Wiley-Interscience publication, New York (1980).

80 (A) L. C. Roy, F. Johnson and William C. Jankowski, Carbon-13 NMR Spectra, published by A/ Wiley-Interscience publication, New York, (1972).

(B) Harold Hart, ^{13}C-NMR, published by Sankyo, Japan (1981).

ABSTRACT

The chemistry, medicinal properties and pharmacological activities of psoralea corylifolica Linn is discussed. Development of commercial method for extraction of bakuchiol and its estimation from seeds are described. Effect of bakuchiol yield with maturity of seeds studied. Abnormal variation in yield of bakuchiol with increasing age of seeds had been observed. This abnormality was found due to abnormal formation of bakuchiol derivatives during long storage of the seed-extract. Derivatives were isolated and identified as (1) 6,7-epoxybakuchiol (2) Δ^{5},7-hydroxybakuchiol Δ^{7},6-hydroxy-bakuchiol. Bakuchiol extracted seeds were used for extraction of psoralen and isopsoralen.