STUDIES ON *ACORUS CALAMUS*, PART II

INVESTIGATION OF VOLATILE OIL

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The capacity to potentiate the sedative action of pentobarbitone by the volatile oil of Indian *Acorus calamus* has been used to screen various fractions of the oil for the presence of the principle responsible. The methods used for the removal of the oxygenated components of the oil did not remove the active material. The results show that the active principle resides in the hydrocarbon fraction of the oil or in an oxygenated component not removed by the methods employed. The volatile oil from the European *Acorus calamus* showed activity similar to the oil from the Indian drug.

THE sedative potentiating principle of *Acorus calamus* L. of Indian origin was shown to be present in the steam-distillable fraction of the light petroleum extract of the rhizomes[1]. Agarwal, Dandiya, Singh and Arora[2] have described other pharmacological properties for a crude alcoholic extract of the rhizomes of *Acorus calamus* L. of Indian origin. Some of these have been shown to be present in the volatile oil[3], and Chopra, Jamwal and Khajuria[4] have reported carminative and antispasmodic properties, and Chopra, Khajuria and Chopra[5] have reported the acute and chronic toxicities of the oil on guinea pigs as well as its antibacterial properties. More recently an extensive pharmacological study of oil of calamus of Indian origin has been made.[6]

The physical and chemical properties of oil of calamus of Indian, Javanese, European, North American, Japanese and Russian origins have been described. The specific gravities of the various oils have been reported and are listed in Table I. The acid number of the various oils lies between 1 and 3 with the ester values ranging between 4 and 12. Thus the oil of calamus of Indian and Javanese origin differs from the others in having comparatively higher specific gravities, lower optical rotations and higher refractive indices. Kelkar and Rao[7] concluded on the basis of their study of the chemical nature of the volatile oil of calamus of Indian origin that the difference between the Indian and the other commercial varieties of the calamus oil was not due to the presence of any new constituents but due to the predominance of asarone in the Indian oil. The Indian oil has been reported to contain 82 per cent asarone while the other commercial varieties have approximately 7 per cent of this constituent. The physical and chemical properties of the oil have been discussed by Guenther.[8] Rao, Sudborough and Watson[9] have reported the presence of 1·5 per cent of oil in calamus of Indian origin.

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TABLE I

PHYSICAL CHARACTERISTICS OF THE VARIOUS OILS

Source	Specific gravity at 15°	Optical rotation	Refractive index at 20°
European	0·959 to 0·974	+ 9° 0′ to +31° 0′	1·5028 to 1·5098
Russian	0·952 to 0·974	+ 9° 39′ to +23° 26′	1·5020 to 1·5289
Japanese	0·973 to 1·023	+ 2° 8′ to +26° 30′	1·5051 to 1·528
Indian	1·069 to 1·081	− 1° 30′ to + 6° 12′	1·5030 to 1·5522
Javanese	1·007 to 1·078	+ 0° 51′ to + 0° 53′	1·5504 to 1·5506
American	0·950 to 0·974	+13° 48′ to +15° 0′	1·5013 to 1·5069

EXPERIMENTAL

Volatile oil from Indian drug. (*a*) 500 g. of rhizomes of *Acorus calamus* were cut small (one-half to one inch) and water distilled[10] using a Florentine receiver for oils heavier than water. The yield of oil was 16·1 ml. The oil was separated and dried over calcium chloride in a desiccator. (*b*) Volatile oil distilled from *Acorus calamus* of Indian origin was obtained from a commercial source (Fritzsche Bros. Inc., New York).

Volatile oil from European drug. This was prepared as described for Indian drug.

The physical properties of these three samples are given in Table II.

TABLE II

PHYSICAL PROPERTIES OF THE THREE SAMPLES OF OIL

	Volatile oil distilled by us from Indian drug	Volatile oil distilled by us from European drug	Volatile oil from Indian drug commercial source
Specific Gravity	1·071 at 18°	0·976 at 18°	1·079 at 25°
Optical rotation	+5·7° at 18°	+12° at 18°	−0° 28′ at 18°
Refractive index	1·5522 at 17°	1·5127 at 17°	1·5522 at 20°
Solubility at 20°	1 vol. in 70 per cent alcohol		1 vol. in 70 per cent alcohol
Acid value			2·0
Saponification value			7·0

Chemical Fractionation

Separation of phenolic compounds. 100 g. of oil was shaken with 300 ml. of N KOH and then allowed to separate overnight. The aqueous layer was separated and made acidic with dilute sulphuric acid and was extracted with three successive portions of chloroform (100, 50 and 50 ml.). The combined chloroform extract was washed with 20 ml. of distilled water and the chloroform removed over a water bath. The residual dark-brown liquid was dried in a vacuum descicator at 80°. The dried residue weighed 0·4 g. It gave a green colouration with ferric chloride solution (see Table V).

Separation of aldehyde. The oil after removal of the phenolic compounds was shaken with 150 ml. of saturated solution of sodium bisulphite. The aqueous layer was separated and treated with a solution of sodium hydroxide until it became alkaline. The liberated oily matter was extracted with successive portions of ether (150, 50 and 50 ml.). The ethereal extract on removal of ether by evaporation, and drying gave a bright golden-yellow viscous residue weighing 0·03 g. (see Table V).

Separation of fractions in succession. (i) *Free acids.* 200 g. of oil was dissolved in ether (500 ml.) and shaken with 50 ml. of 3 per cent sodium carbonate solution. The aqueous layer was separated and acidified with dilute sulphuric acid. An oily layer which separated was extracted with ether. The residue, weighing 0·3 g., after evaporation of ether, melted at 28°.

(ii) *Phenols.* The etheral solution of oil was shaken with 100 ml. of 2 per cent aqueous KOH. The liberated phenol weighing about 0·7 g. gave a blue colouration with solution of ferric chloride. It melted at 26°.

(iii) *Acids present as esters.* The ether was removed and the residual oil was refluxed on the water bath with 5 per cent alcoholic KOH (80 ml.) for 3 hours. The acids which separated on acidifying the alcoholic layer were in the form of a semi-solid residue weighing 0·5 g.

(iv) *Aldehydes.* The oil after treatment with alcoholic KOH was shaken with 80 ml. of saturated solution of potassium metabisulphite. The aqueous layer on acidification yielded an oil which was extracted with ether. The ethereal extract on evaporation yielded a solid residue (weight 0·056 g.) which could be crystallised from absolute ethanol. The crystalline material melted at 190–210°.

(v) *Alcohols.* The residual oil after treatment as described above, was dried over anhydrous sodium sulphate and dissolved in 200 ml. of dry ether. The ethereal solution was shaken with small pieces of metallic sodium. The reaction was allowed to take place for 24 hours, after which the solution was extracted with three portions (100, 50 and 50 ml.) of water. The combined aqueous extract was made acidic with dilute hydrochloric acid and then extracted with chloroform. The chloroform extract was evaporated to dryness and the semi-solid residue weighed 0·4 g.

The following fractions of the oil were collected and reserved for pharmacological testing: (A) free acids, (B) phenols, (C) acids present as esters, (D) aldehydes, (E) alcohols, (F) oil treated to remove free acids, (G) oil treated to remove acids and phenols, (H) oil treated to remove free acids, phenols and acids present as esters, (I) oil treated to remove free acids, phenols, acids present as esters, aldehydes, (J) oil treated to remove total acids, phenols, aldehydes and alcohols (see Table VI).

Pharmacological Observations

Acute toxicity. The LD50 was assessed using doses varying between 0·12 to 0·22 g./kg. of the volatile oil which were injected intraperitoneally into male mice weighing between 20 to 40 g. for acute toxicological studies. The mortality was recorded 24 hours after the injection (see Table III).

Potentiation of sedative activity. Male, white, mice weighing between 20 to 40 g. were used. Each mouse was weighed and a dose of the drug directly proportional to the weight of the mouse was injected intraperitoneally. After 30 minutes from the time of injection of the drug, pentobarbitone sodium, 30 mg./kg. body weight was injected intraperitoneally

using a 0·3 per cent solution. The same dose of barbiturate was given to a group of control animals to which no drug had been administered earlier. The number of mice which lost their righting reflex was recorded.

Oils from different sources were tested according to the method given. In all cases dilutions of 1 to 50 (w/v) in water containing 3 per cent Tween 80 were employed (see Table IV).

The potentiation action of the other fractions was also studied using this procedure (see Table VI).

Results and Discussion

The physical properties determined for the volatile oil from various sources are recorded in Table II. The physical properties of the volatile oil of the Indian *Acorus calamus* distilled in our laboratories and that obtained from a commercial source had similar physical properties. The volatile oil from the European drug differed in its physical properties, having a lower specific gravity, higher optical rotation and a lower refractive index than the other two samples of the oil. However, there appeared to be no significant difference in the potentiation action of these three samples of oil on the sedative property of pentobarbitone sodium as indicated by the results in Table IV.

Table VI indicates that fractions separated by means of chemical fractionation did not show any appreciable potentiating activity on the sedative action of pentobarbitone sodium (A to E). Treatment of the oil to remove phenols and aldehydes and some of the related carbonyl compounds, however, did cause an appreciable increase in this activity of the oil (I and J). The increase was such that only half the dose of the oil was required for the same potentiation action (Table V and Table VI, I and J). Since the volume of these two fractions represents less than 1 per cent of the total volume of the oil this increase could not be explained on the basis of an increase in concentration due to the removal of inert material. The removal of only one of these fractions did not result in an increase or enhancement of activity (Table V, Table VI, compare H, I, J). It appears that the presence of both of these fractions in the oil causes an antagonistic action to the potentiation of the sedative property of the volatile oil of *Acorus calamus*.

The results obtained appear to indicate that the potentiation activity resides in the hydrocarbon fraction of the oil or in an oxygenated component not removed by the methods employed. It seems unlikely that asarone (1:2:5-trimethoxy-4-propenyl benzene) is responsible for the activity evidenced by this oil since it is present to the extent of approximately 80 per cent in the Indian oil and about 7 per cent in the European oil. It has been already stated that the results obtained indicated no appreciable difference in the sedative potentiation action of these oils.

The acute toxicity of the Indian oil is given in Table III. The dose estimated to cause 50 per cent mortality was 0·177 g./kg. It will be observed from Table V that the elimination of the phenolic and aldehydic fractions from the oil resulted in an increase in the toxicity of the oil as well as in the sedative potentiation activity of the remaining oil.

TABLE III

ACUTE TOXICOLOGICAL STUDIES OF VOLATILE OIL OF *Acorus calamus* ON WHITE MICE

Dose of the volatile oil	No. of mice injected	Mortality up till 24 hours	Mortality per cent
0·12 g./kg.	20	nil	0
0·14 g./kg.	20	1	5
0·16 g./kg.	20	4	20
0·18 g./kg.	20	13	52
0·20 g./kg.	20	18	90
0·22 g./kg.	20	20	100

TABLE IV

POTENTIATION ACTION OF VOLATILE OIL OF *Acorus calamus* FROM DIFFERENT SOURCES, ON THE SEDATIVE ACTION OF PENTOBARBITONE SODIUM 30 mg./kg.

Source of oil	Dose g./kg.	No. of mice injected	No. of deaths	No. which lost righting reflex
Volatile oil from Indian drug distilled in our laboratory	0·1	20	nil	19
Volatile oil from Indian drug, commercial source	0·1	20	nil	20
Volatile oil from European drug distilled in our laboratory	0·1	20	nil	18

TABLE V

POTENTIATION ACTION OF PHENOLIC AND ALDEHYDIC FRACTIONS OF VOLATILE OIL OF *Acorus calamus* ON THE SEDATIVE ACTION OF PENTOBARBITONE SODIUM 30 mg./kg.

Fraction	Dose g./kg.	No. of mice injected	No. of deaths	No. which lost righting reflex
Phenolic	0·1	20	nil	3
Aldehydic	0·1	10	nil	4
Oil devoid of phenolic fraction ..	0·1	20	nil	19
Oil devoid of phenolic and aldehydic fraction	0·1	5	5	
Oil devoid of phenolic and aldehydic fraction	0·05	15	nil	15
Control	nil	10	nil	nil

TABLE VI

POTENTIATION ACTION OF SUCCESSIVE FRACTIONS OF VOLATILE OIL OF *Acorus calamus* ON THE SEDATIVE ACTION OF PENTOBARBITONE SODIUM 30 mg./kg.

Fraction	Dose g./kg.	No. of mice injected	No. of deaths	No. which lost righting reflex
A	0·1	10	nil	nil
B	0·1	10	nil	1
C	0·1	10	nil	nil
D	0·1	10	nil	nil
E	0·1	10	nil	1
F	0·1	10	nil	10
G	0·1	10	nil	9
H	0·1	10	nil	9
I	0·1	10	9 died before pentobarbitone sodium was given	
I	0·05	10	nil	9
J	0·05	10	nil	10

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