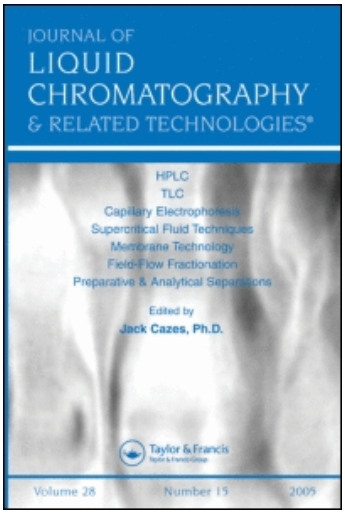

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G. Sen[a]; N. B. Mulchandani[a]; A. V. Vyas[a]
[a] Bio-Organic Division Bhabha Atomic Research Centre Trombay, Bombay, (INDIA)

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REVERSED PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHIC SEPARATION OF PRECOCENES-I, -II, ANTIJUVENILE HORMONES AND THEIR DERIVATIVES

G. SEN, N.B. MULCHANDANI, AND A.V. VYAS

Bio-Organic Division
Bhabha Atomic Research Centre
Trombay, Bombay-400 085, (INDIA).

ABSTRACT

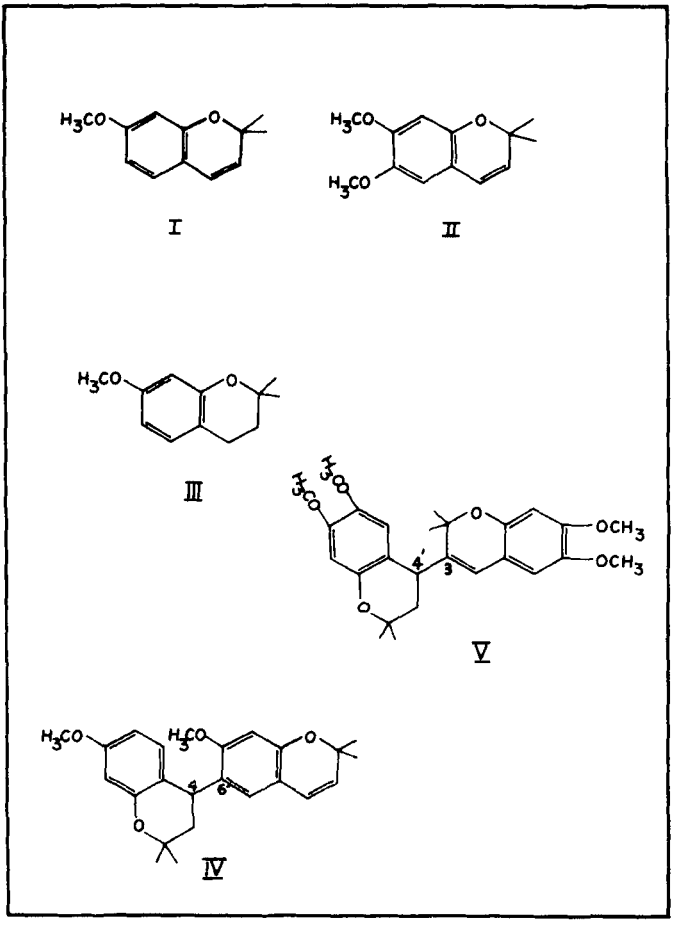
HPLC separation of precocene-I, precocene-II, dihydroprecocene-I, precocene-I dimer and precocene-II dimer has been carried out under varying conditions of eluent concentration for MeOH: H_2O system. It is observed that the mixture of above compounds is adequately resolved using MeOH: H_2O (3:1).

INTRODUCTION

Certain chromene derivatives namely precocenes-I (I), and -II (II) isolated from the plants of genus Ageratum have been found to exhibit antijuvenile hormone activity (1). These have been termed as precocenes due to their ability to bring about precocious metamorphosis in young larval stages of the test insects, resulting thereby into unviable or moribund miniature adults. Precocenes contribute a new type of insect growth regulators and have been referred to as fourth generation insecticides. It has been suggested that the epoxides of precocenes formed during metabolic activity might be the species responsible for their anti-JH activity (2). Therefore the study of mode of action of these compounds and anti-JH activity of derivatives of these compounds becomes very important. However in the biological work as stated above one always comes across nanogram amounts of the metabolites. The detection of

these by any other analytical tool would be extremely difficult. Hence, the separation and detection of these compounds by HPLC technique becomes indispensable.

The five precocenes, namely precocene-I, precocene-II, dihydro-precocene-I (III), precocene-I dimer (7, 7'-dimethoxy, 2,2,2', 2'-tetramethyl-3' (4')-dihydro 6'-4-bichroman (IV) and precocene-II dimer (6,7,6',7'-tetramethoxy-2,2,2',2' tetramethyl-3' (4')-dihydro 3-4' S-bichroman) (V) were chosen as model compounds for their separation studies. These were found to exhibit a reasonably strong UV absorption and could be detected in low concentration, using a UV detector (280 nm).



MATERIALS AND METHODS

Chemicals and Reagents

Precocenes-I and -II were isolated from Ageratum conyzoides plants (3). Precocene-II dimer was synthesized by methods reported earlier (3), while, precocene-I dimer was synthesized by an alternative method (4). All of these were dissolved in methanol. Methanol (spectroscopic grade) and acetone (analytical reagent grade) were purchased from E. Merck (India).

Apparatus

A Water:Associates Instrument, Model ALC/GPC 244, equipped with a Model 6000 A solvent delivery system, U6K injector and Model 440 detector was used.

A μ Bondapak C_{18} column (stainless steel 300 mm x 3.9 mm I.D.) with particle size (10 microns) was purchased from Water:Associates. The dead volume of column between the point of injection and the UV detector was found using acetone.

Analytical conditions

Various mixtures of methanol and water were used as mobile phases. One pump was employed to pump water and the other for methanol, the percentage of each being controlled by the programmer. Prior to the analysis, the column was washed for half an hour with methanol (flow rate 1 ml/min.).

RESULTS

The separation of precocenes and their derivatives was carried out with good resolution using μ Bondapak C_{18} column and MeOH: H_2O eluent system. Fig. 1 shows separation for MeOH:H_2O (3:1) eluent system. The retention times (R_t), capacity factors (k') and separation factors (α) for the above eluent system are recorded in Table 1. The capacity factors are calculated with acetone as the reference, as it absorbs in the UV and has no retention on the column.

Similarly, these compounds showed clean separation with CH_3CN: H_2O (3:2) as eluent system. The retention times (R_t), capacity factors (k') and separation factors (α) are shown in Table 2.

Effect of solvent strength on retention time.

The variation of retention time versus the polarity of the eluent was also investigated. Five concentrations of the eluent methanol and

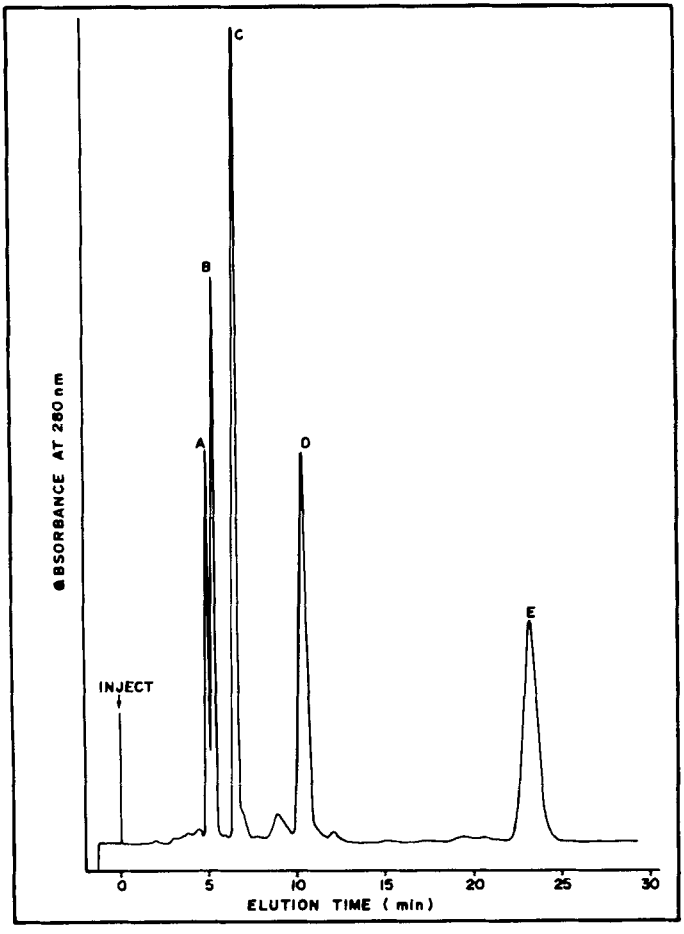


FIGURE 1 :- HPLC separation of precocene-I, precocene-II, dihydro precocene-I and dimers of precocene-I and -II at 280 nm with a μBondapak C_{18} column and MeOH:H_2O (3:1) at a flow rate of 1 ml/min. Chromatogram represents a standard solution containing known quantities of the following in the 20λinjection : Precocene-I, 4 μg (C), precocene-II, 5 μg (B), dihydroprecocene-I, 4 μg (A), dimer of precocene-I, 10 μg (E) and dimer of precocene-II, 7 μg (D).

TABLE I

RETENTION TIMES, CAPACITY FACTORS AND SEPARATION FACTORS FOR PRECOCENES USING MeOH:H_2O (3:1 V/V) AS ELUENT. FLOW RATE = 1 ml/min. DEAD TIME = 3.25 min.

Precocene	Retention Time (min)	k'	α
Dihydro-precocene-I	5.0	0.54	1.17
Precocene-II	5.3	0.63	1.46
Precocene-I	6.25	0.92	2.26
Precocene-II-dimer	10.0	2.08	2.92
Precocene-I-dimer	23.0	6.08	

TABLE II

RETENTION TIMES, CAPACITY FACTORS AND SEPARATION FACTORS FOR PRECOCENES USING H_2O:CH_3CN (2:3 V/V) AS ELUENT. FLOW RATE = 1 ml/min. DEAD TIME = 3.25 min.

Precocenes	Retention Time (min.)	k'	α
Dihydro precocene-I	5.02	0.54	1.5
Precocene-II	5.9	0.81	1.56
Precocene-I	7.38	1.27	1.81
Dimer of precocene-II	10.72	2.23	2.71
Dimer of precocene-I	23.52	6.24	

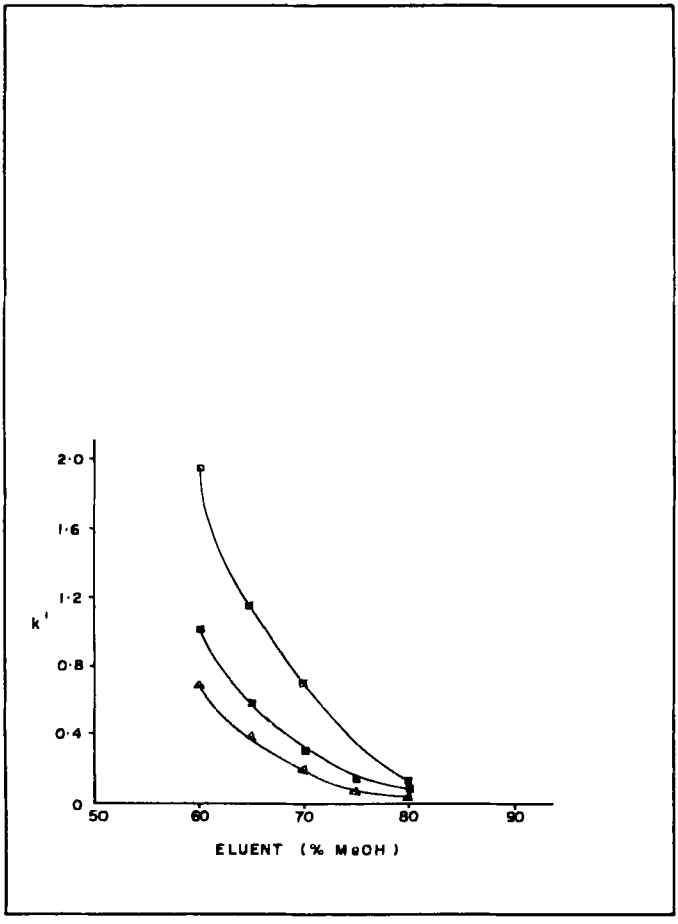


FIGURE 2 :- Effect of polarity of eluent on the capacity factor of precocenes, at a flow rate of 1.5 ml/min. The symbols in the figure are: -□- = I, -■- =II, -△- =III.

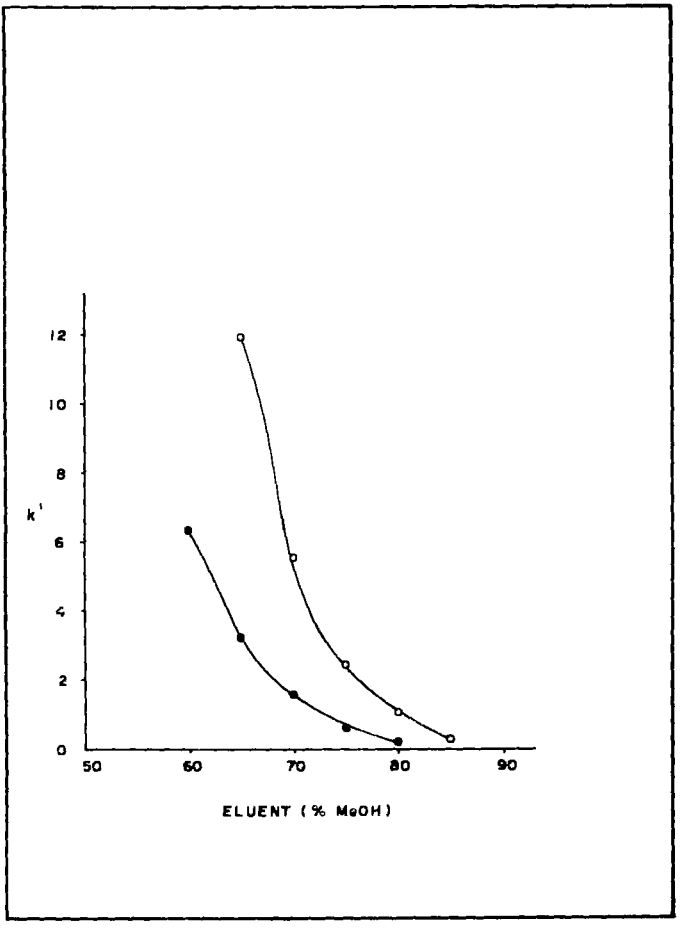


FIGURE 3 :- Effect of polarity of eluent on the capacity factor of precocene dimers at a flow rate of 2 ml/min. The symbols in the figure are:

-○- = IV, -●- = V.

water (20, 25, 30, 35, 40) were taken for this purpose. The capacity factors of these compounds were calculated and are plotted against polarity of the eluent (Figs. 2 and 3). It was found that k' decreased with increasing concentration of methanol in the eluent.

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