Journal of Chromatography, 198 (1980) 203–206
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,971

Note

Reversed-phase high-performance liquid chromatographic separation and analysis of some physalins (13,14-seco-16,24-cyclo-steroids)

G. SEN, N. B. MULCHANDANI* and A. V. PATANKAR
Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085 (India)
(First received February 26th, 1980; revised manuscript received May 28th, 1980)

Physalins are a novel class of natural products, having 13,14-*seco*-16,24-cyclo-steroid structures. They are potential anticancer agents, since they contain the methylene lactone system observed in many sesquiterpene lactones[1]. Also, they have been shown to possess anti-inflammatory and anti-arthritic activities[2]. These compounds have similar structures and hence their separation by the usual techniques is difficult. We have been successful in the separation of physalins by high-performance liquid chromatography (HPLC).

The six physalins A (I), B (II), B-epoxide (III), D (5,6-dihydroxyphysalin B) (IV), E (V) and H (VI) were chosen for this work. They were found to exhibit reasonably strong UV absorption and could be detected in low concentration, using a UV detector (254 nm).

EXPERIMENTAL

Chemicals and reagents

Physalins B, B-epoxide and D were isolated from *Physalis minima* plants and characterized by Mulchandani *et al.*[3]. The samples of physalins A, E and H were kindly provided by Professors T. Matsuura and L. Ramachandra Row, respectively. All of these were dissolved in methanol. Methanol (spectroscopic grade) and acetone (analytical reagent grade) were purchased from E. Merck (India).

Apparatus

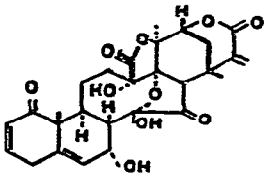
A Waters Assoc. Model ALC/GPC 244 chromatograph, equipped with a Model 6000 A solvent-delivery system, U6K injector and Model 440 detector, was used. A μBondapak C_{18} column (stainless steel, 300 × 3.9 mm I.D.) with particle size 10 μm was purchased from Waters Assoc. (Milford, MA, U.S.A.).

Aanalytical conditions

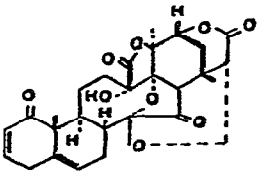
Various mixtures of methanol and water were used as mobile phases. One pump was employed to pump water and the other for methanol, the percentage of each being controlled by the programmer.

The flow-rate of the eluent was kept constant at 1 ml/min unless otherwise stated. Prior to the analysis, the column was washed for 30 min with methanol (flow-rate 1 ml/min).

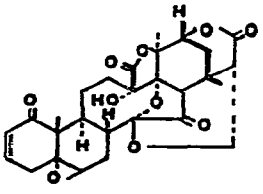
0021-9673/80/0000–0000/$02.25



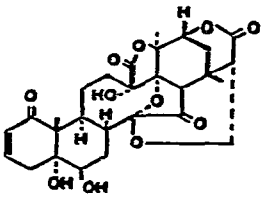
I



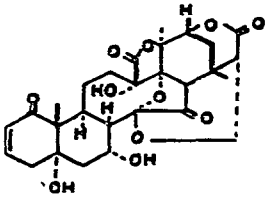
II



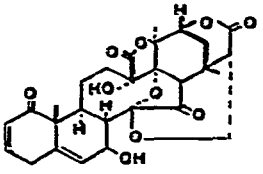
III



IV



V



VI

RESULTS AND DISCUSSION

In order to establish appropriate conditions for separation, HPLC was carried out under different conditions. The retention times for all the six physalins on a μBondapak C_{18} column with water–methanol (2:3) as eluent are shown in Table I. The capacity factors are calculated with acetone as the reference, as it absorbs in the UV and has no retention on the column.

Study of detector response versus physalin concentration

The calibration curves for the six physalins plotting peak height *versus* the physalin concentration were linear.

Effect of solvent strength on retention time

The variation of retention time *versus* polarity of the eluent was also investigated. Six concentrations of the eluent, 15, 20, 25, 30, 40 and 50% water in methanol, were taken for this purpose. The percentage of methanol in the eluent *versus* retention times of the six physalins was studied. It was observed that there is an exponential decrease of retention time with increasing concentration of methanol in the eluent.

TABLE I

RETENTION TIMES AND CAPACITY FACTORS OF PHYSALINS ON A μBONDAPAK C_{18} COLUMN

Eluent: water–methanol (2:3); flow-rate, 1 ml/min. Dead volume, 3.5 min.

Sample	*Retention time (min)*	*k'*
Physalin A	5.12	0.46
Physalin B	11.61	2.32
Epoxyphysalin B	6.3	0.8
Physalin D	4.53	0.29
Physalin E	4.63	0.32
Physalin H	6.01	0.72

The capacity factor, k', of the physalins was calculated and is plotted against the polarity of the eluent in Fig. 1. The variation of k' *versus* the polarity of the eluent is also exponential. The capacity factor of physalins decreases with increasing concentration of methanol in the eluent.

Effect of flow-rate on retention time

The relationship between retention time and flow-rate from 1 to 2.5 ml/min was studied. In this case water–methanol (2:3) was used as the eluent. The retention times decreased as the flow-rate increased.

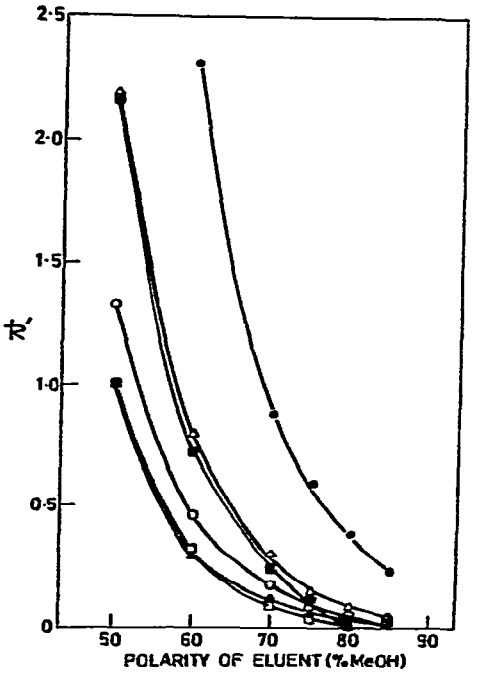


Fig. 1. Effect of polarity of the eluent on the capacity factor of physalins at 254 nm with a μBondapack C_{18} column at a flow-rate of 1 ml/min. Physalins: I (○); II (●); III (△); IV (▲); V (□); VI (■).

ACKNOWLEDGEMENTS

We thank Professor Teruo Matsuura, Department of Synthetic Chemistry, Faculty of Engineering, Toshida, Kyoto, Japan, for the sample of physalin A and Professor L. Ramachandra Row, Department of Chemistry, Andhra University, Waltair, India, for the samples of physalins E and H.

REFERENCES

1 I. H. Hall, K-H. Lee, C. O. Starnes, S. A. Eigebaly, T. Ibuka, Y-S. Wu, T. Kimura and M. Haruna, *J. Pharm. Sci.*, 67 (1978) 1235.
2 P. D. Sethi, *Second Conference, Commonwealth Pharmaceutical Association, Bombay, India, January 1977*, Abstract C18.
3 N. B. Mulchandani, S. S. Iyer and L. P. Badheka, *Planta Med.*, 37 (1979) 268.