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Note

Retention behavior of selected colchicine derivatives on reversed-phase high-performance liquid chromatographic systems

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Colchicine (I), the major alkaloid of *Colchicum* species, is used in the treatment of gout, and, together with colchicine derivatives, are of interest as potential antineoplastic agents[1]. We are currently examining the use of microorganisms to prepare metabolically derivatives of colchicine[2]. Additionally, we are examining colchiceine (II) and its derivatives as potential microbial metabolites of colchicine[3–5].

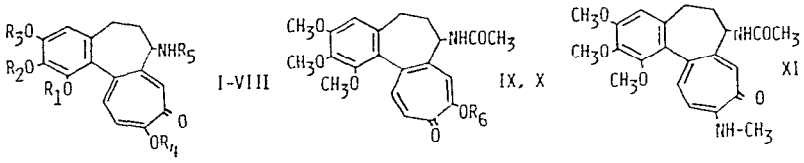

Fig. 1. Structural formulas of colchicines and colchiceine. I: R_1 to $R_4 = CH_3$, $R_5 = COCH_3$; II: R_1 to $R_3 = CH_3$, $R_4 = H$, $R_5 = COCH_3$; III: R_1 to $R_4 = CH_3$, $R_5 = H$; IV: R_1 to $R_5 = CH_3$; V: R_1, R_2, $R_4 = CH_3$, $R_3 = H$, $R_5 = COCH_3$; VI: R_1, R_3, $R_4 = CH_3$, $R_2 = H$, $R_3 = COCH_3$; VII: R_2, R_3, $R_4 = CH_3$, $R_1 = H$, $R_5 = COCH_3$; VIII: R_1 to $R_3 = CH_3$, $R_4 = C_2H_5$, $R_5 = COCH_3$; IX: $R_6 = CH_3$; X: $R_6 = C_2H_5$.

In order to analyze and quantify colchicine and five colchicine derivatives in microbiological systems, we have recently described a selective high-performance liquid chromatographic (HPLC) procedure[6]. We have also reported a derivatization technique that enables colchiceine (II) to be determined in the presence of colchicine (I) by HPLC[7]. In complex mixtures, it was discovered that certain derivatives of I co-chromatographed, and further studies were initiated to determine the retention behavior of a variety of colchicines with different reversed-phase HPLC systems. In the present work the retention behavior of colchicine and nine of its derivatives was evaluated for ternary mobile phase systems (*i.e.* acetonitrile–methanol–buffer) of varying concentration. The derivatives studied include the N-desacetylcolchicine (III), N-desacetyl-N-methylcolchicine (*i.e.* demecolcine) (IV), 3-demethylcolchicine (V), 2-demethylcolchicine (VI), 1-demethylcolchicine (VII), ethylcolchicinate (VIII), isocolchicine (IX), ethylisocolchicinate (X), and N-methylcolchiceinamide (XI). Four

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reversed-phase columns were compared for their relative retention behavior and conclusions were drawn as to the most suitable HPLC system for rapid identification of these compounds.

EXPERIMENTAL

HPLC system

A Model 950 pump and 970A variable-wavelength detector (Tracor, Austin, TX, U.S.A.) with a Model 7120 100-μl loop injector (Rheodyne, Berkeley, CA, U.S.A.) were employed for all analyses. Detection was at 350 nm, and a Model HP-3380A reporting integrator (Hewlett-Packard, Palo Alto, CA, U.S.A.) at an input sensitivity of 0.1 V/a.u. and a slope sensitivity of 1 mV/min was used for peak area measurement and chromatogram recording. The flow-rate was constant at 2.0 or 3.0 ml/min. μBondapak C_{18} and phenyl columns were obtained from Waters Assoc. (Milford, MA, U.S.A.) and LiChrosorb RP-18 and RP-8 columns from Alitech (Arlington Heights, IL, U.S.A.) Dead time (t_0) was measured by the pressure fluctuation observed on the baseline after an injection of mobile phase. Analyses were performed with methanol–acetonitrile–phosphate buffer mobile phases of varying composition.

Reagents

Organic solvents used in the mobile phase were chromatographic quality (LiChrosorb; MCB, Cincinnati, OH, U.S.A.). Water was deionized and doubly distilled in glass. Mobile phases were prepared by filtering individual solvents through glass fiber pads, GF/F grade (Whatman, Clifton, NJ, U.S.A.) mixing and degassing by sonication prior to use.

Standard compounds

Colchicine (I) and N-methylcolchiceinamide (XI) were purchased from Aldrich (Milwaukee, WI, U.S.A.). Colchiceine (II) was prepared as described[7] by the mild acid treatment of colchicine according to the method of Zeisel[8], and was identical to a sample provided by T. J. Fitzgerald of Florida A. & M. University (Tallahassee, FL, U.S.A.). Ethylisocolchicinate (X) and ethylcolchicinate (VIII) and isocolchicine (IX) were prepared as described in ref. 7.

Samples of colchicine derivatives III–VII were kindly provided by Dr. J. A. R. Mead and Dr. A. Brossi of the National Institutes of Health (Bethesda, MD, U.S.A.). All standard compounds were homogenous as determined by thin-layer chromatography (TLC) and HPLC.

RESULTS AND DISCUSSION

The retention behavior of colchicine derivatives I and III–XI were studied to select an appropriate HPLC system. Considerable variation in capacity ratios was observed with different reversed-phase packings when operating with our reported mobile phase[6], as shown in Table I. A 30-cm μBondapak C_{18} column was able to separate all ten compounds, but the adjusted retention time for the last solute, N-methylcolchiceinamide (XI), was greater than 30 min at a flow-rate of 3 ml/min. By comparison, a shorter 15-cm μBondapak–phenyl column eluted this compound more

TABLE I

RETENTION BEHAVIOR OF COLCHICINE DERIVATIVES ON REVERSED-PHASE SILICA GEL COLUMNS

Acetonitrile–methanol–phosphate buffer (pH 6, 0.022 *M*) (16:5:79) as mobile phase. Each packing particle size = 10 μm.

Compound		*Capacity ratio (k')*			
		*Column**			
		A	*B*	*C*	*D*
3-Demethylcolchicine	(V)	4.1	—	3.6	5.4
2-Demethylcolchicine	(VI)	4.8	3.9	4.1	6.3
N-Desacetylcolchicine	(III)	7.1	—	6.1	10.1
1-Demethylcolchicine	(VII)	9.4	—	7.9	14.8
Demecolcine	(IV)	10.7	—	9.4	14.8
Colchicine	(I)	14.2	10.0	11.2	21.1
Ethylisocolchicinate	(X)	18.2	10.7	14.4	27.1
Ethylcolchicinate	(VIII)	26.0	15.7	19.8	43.1
N-Methylcolchiceinamide	(XI)	38.5	21.1	27.4	60.2
t_0 (min) at 3 ml/min flow-rate		0.87	0.69	1.2	0.38

* A = μBondapak C_{18} (30 cm); B = μBondapak–phenyl (15 cm); C = LiChrosorb RP-8 (25 cm); D = LiChrosorb RP-18 (10 cm).

quickly, but with some loss in resolution between colchicine and ethylisocolchicinate (I and X). A 25-cm LiChrosorb RP-8 column produced a similar but slightly more time consuming separation for most solutes compared to that with the μBondapak C_{18} column, while a 10-cm LiChrosorb RP-18 column was unable to separate 1-demethylcolchicine (VII) from demecolcine (IV) but eluted all solutes within 22 min. This short column was able to separate completely the ethyl isomers (VIII and X) and N-methylcolchiceinamide (XI) within 16 min at a flow-rate of 4 ml/min.

Variations in capacity ratio were also observed when the composition of the mobile phase was changed slightly, as shown for the μBondapak C_{18} column in Fig. 2A–C. A change of 1 % in the acetonitrile fraction in the mobile phase produced dramatic changes in the retentions of all of the colchicine derivatives (Fig. 2A), while an equivalent change in the methanol fraction did not alter the capacity ratios as sharply (Fig. 2B). The retentions of the easily ionizable colchicine derivatives III and IV showed major changes as the pH of the phosphate buffer was altered (Fig. 2C), as had previously been noticed[6]. These two compounds were separated most completely from the other compounds at pH 6 as indicated in Fig. 2C and in Fig. 3. The non-ionizable colchicine derivatives showed only slight variations in retention with changes in buffer pH.

The μBondapak C_{18} column provided the best separation for all ten colchicine derivatives with the acetonitrile–methanol–phosphate buffer (pH 6) (16:5:79) mobile phase, as shown in Fig. 3, for a flow-rate of 3 ml/min. The N-methylcolchiceinamide peak, which is not included in the figure, eluted at 34.4 min. This system has been chosen for the analysis of colchicine using demecolcine as the internal standard because of its resolving power[7]. Isocolchicine (IX) was the sole colchicine derivative that was not completely resolved from the internal standard with this system. It is

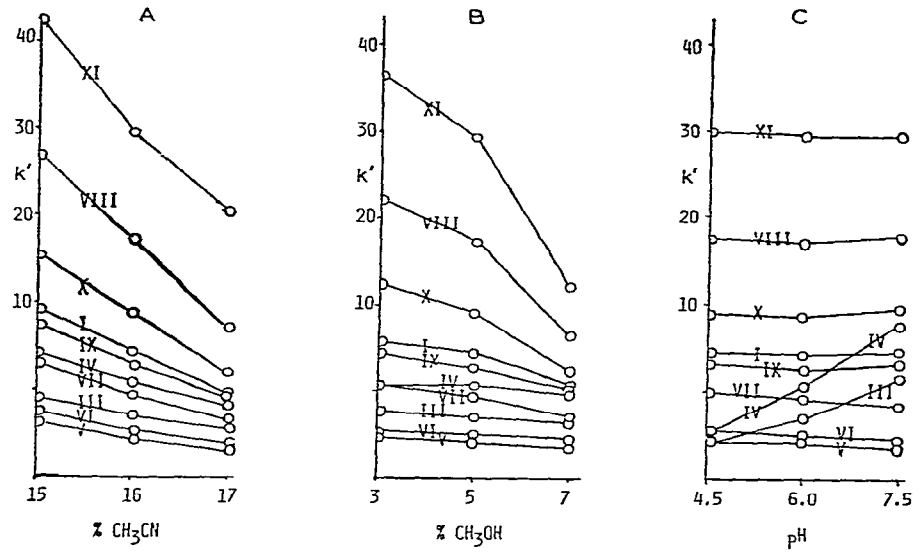

Fig. 2. Capacity ratio (k') as a function of composition of the ternary mobile phase. Compound numbers correspond to those described in Fig. 1. (A), Variation of acetonitrile concentration from 15–17% (phosphate buffer pH 6, 80–78%) with methanol constant at 5%; (B), variation of methanol concentration from 3–7% (phosphate buffer pH 6, 81–77%) with acetonitrile constant at 16%; (C), variation in pH of the phosphate buffer ($\mu = 0.05, 0.022$ M in all cases) in a mobile phase composed of methanol–acetonitrile–buffer (5:16:79).

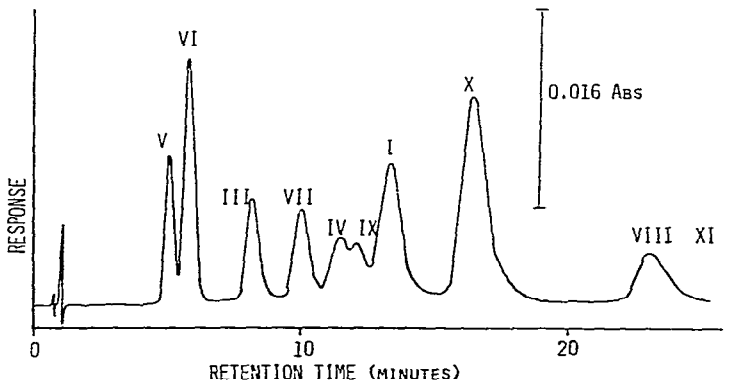

Fig. 3. HPLC separation of colchicine (I) and derivatives (III–X) on a 30-cm μBondapak C_{18} column with methanol–acetonitrile–phosphate buffer (pH 6) (5:16:79). The compound numbers correspond to those in Fig. 1.

inconceivable, however, that this compound would be present as a metabolite of colchicine, and also would not be chosen as a potential internal standard. The iso derivatives IX and X eluted before the corresponding normal-colchicines I and VIII on all the reversed-phase packings studied. The increased polarity of the iso series is also consistent with TLC mobility on silica adsorbents[6].

The shorter reversed-phase columns listed in Table I do have utility in specific analyses, and have proved especially useful for the rapid and complete separation of the ethyl derivatives VIII and X from colchicine. The 10-cm LiChrosorb RP-18 column is now being employed for the rapid analysis of colchiceine (II) as its ethylated derivatives (VIII and X) in the presence of colchicine in microbial systems[7]. It is hoped that the systems described herein will aid others in specific applications relating to the HPLC analysis of colchicine and its derivatives.

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REFERENCES

1 A. C. Sartorelli and W. A. Creasey, *Ann. Rev. Pharmacol.*, 9 (1969) 51.
2 P. J. Davis, *Antimicrob. Ag. Chemother.*, (1981) in press.
3 K. Kieslich, *Microbial Transformations of Non-Steroid Cyclic Compounds*, G. Thieme, Stuttgart, 1976, p. 225.
4 L. Velluz and P. Bellet, *C.R. Acad. Sci. (Paris), Ser. C.*, 248 (1959) 3453.
5 Roussel-UCLAF, *British Pat.* 923,421 (1963); *C.A.*, 13320a (1963).
6 P. J. Davis and A. E. Klein, *J. Chromatogr.*, 188 (1980) 280.
7 A. E. Klein and P. J. Davis, *Anal. Chem.*, 52 (1980) 2432.
8 S. Zeisel, *Monatsh. Chem.*, 9 (1888) 1.