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Study of cytokinin separation using capillary electrophoresis with cyclodextrin additives

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Abstract

The separation of a set of 10 cytokinins by capillary electrophoresis was investigated. The influence of various cyclodextrin derivatives (α-, β-, γ-, hydroxypropyl-β- and dimethyl-β-cyclodextrins) in the background electrolyte on the migration characteristics of the compounds investigated was studied. The relationship between the structures of single cytokinins and the type of cyclodextrin used was evaluated. A set of all 10 cytokinins was successfully separated in 100 mmol l^{-1} phosphate–Tris buffer (pH 2.5) as a background electrolyte under different experimental conditions e.g., with addition of 25 mmol l^{-1} γ-cyclodextrin. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Background electrolyte composition; Purine; Adenine; Cytokinins; Plant hormones

1. Introduction

The investigation of plant hormones (phytohormones) is one of the most important research activities of contemporary biology. These substances regulate cell division as promoters (auxins, giberelins, cytokinins) or inhibitors (abscisic acid). Cytokinins, as representatives of the first group of phytohormones, support cell division, bud formation and stem branching. Cytokinins stimulate differentiation of plant tissues. Some of these derivatives can effectively reduce the growth of some types of mammal tumors [1–3]. The structure of all cytokinins is derived from adenine or adenosine by the substitution of one hydrogen atom in the amino group in position 6 of the purine system (see Table 1) [4].

Immunoanalytical as well as chromatographic methods have been successfully used for analysis of cytokinins. Immunomethods, like enzyme-linked immunosorbent assay (ELISA) [5] and/or radioimmunoassay (RIA) [6,7] serve to determine individual cytokinins in very low concentrations and in combination with suitable separation techniques [for example high-performance liquid chromatography (HPLC)–ELISA] [5,8] offer robust methods for routine analyses. In the field of separation techniques, HPLC [9–11] and ion-exchange chromatography [12,13] are the most often used methods for determination of cytokinins but the use of gas chromatography–mass spectrometry (GC–MS) [14] has also been described. Capillary electrophoresis (CE) is a very promising analytical technique for the

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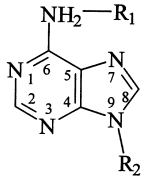
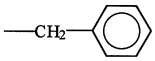
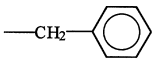
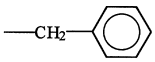
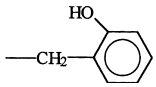
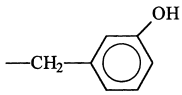
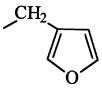
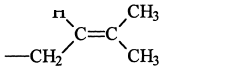
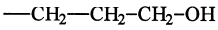
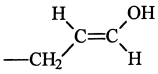
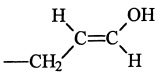
Table 1
Names and structures of discussed cytokinins



Abbreviation	Name	R_1	R_2
BAP	Benzylaminopurine		–H
BAPG	Benzylaminopurineglukoside		β-*O*-Glucopyranosyl
BAPR	Benzylaminopurineriboside		β-*O*-Ribofuranosyl
OTR	*ortho*-Topolineriboside		β-*O*-Ribofuranosyl
MTR	*meta*-Topolineriboside		β-*O*-Ribofuranosyl
KR	Kinetineriboside		β-*O*-Ribofuranosyl
ZR	Zeatineriboside		β-*O*-Ribofuranosyl
DHZR	Dihydrozeatineriboside		β-*O*-Ribofuranosyl
IP	Isopentenyladenine		–H
IPR	Isopentenyladenosine		β-*O*-Ribofuranosyl

separation of cytokinins [15] and other purine derivatives [16–18]. In comparison with HPLC (an expensive and time consuming process) or GC (derivatization before analysis required) CE offers higher separation efficiency, shorter analytical time and lower costs.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of several glucopyranose units. Their utilization in analytical chemistry has been continuously growing. They are very popular especially as chiral selectors [19,20] but their unique capability to form inclusion complexes is also very useful in an achiral environment [21].

The aim of this work was to investigate the influence of different types of CDs on the migration behavior of a studied set of 10 cytokinins.

2. Experimental

Capillary zone electrophoresis (CZE) separation was performed in an uncoated fused-silica capillary [47 cm (effective length 40 cm)×50 μm] at an electric field of 426 V cm^{-1} (current about 50 μA). A P/ACE 5510 (Beckman Instruments, Fullerton, CA, USA) with diode array detector (λ=190–320 nm) was used. One hundred mmol l^{-1} phosphate–

Tris buffer, pH 2.5 was used as a background electrolyte (BGE). The BGE was modified by addition of α-, β-, γ-cyclodextrin, hydroxypropyl-β-cyclodextrin (HP-β-CD) or dimethyl-β-cyclodextrin (diMe-β-CD). With regard to absence of electroosmotic flow (EOF), only the BGE with CD addition was loaded onto the capillary.

All samples were prepared in deionized water at a concentration of 10^{-4} mol l^{-1}. Mobility of each cytokinin was measured in separate runs.

Phosphoric acid and acetone (analytical grade) were purchased from Lachema (Brno, Czech Republic). All CD derivatives, except α-CD, were obtained from Beckman Instruments; α-CD and tris-(hydroxymethyl)aminomethane were from Merck (Darmstadt, Germany). Commercially available cytokinins were purchased from Sigma–Aldrich (St. Louis, MO, USA), new derivatives (OTR, MTR) were obtained from Institute of Experimental Botany, Academy of Science of Czech Republic (Olomouc, Czech Republic).

3. Results and discussion

Purine bases have pK_a values around 4.2 (dissociation constant of conjugated acid) [15]. Purine glycosides are a little bit more acidic (pK_a~3). That is why acid buffer (pH 2.5) was chosen. The elimination of EOF is a further advantage of an acidic medium. The EOF was measured by acetone and no signal was observed until 120 min (effective mobility less than $1.3 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}).

CDs can interact with different parts of a molecule accordingly to their dimensions and chemical structure. Internal diameters of the hydrophobic CD cavities formed from 6 (α-CD), 7 (β-CD) and 8 (γ-CD) glucopyranose units are on average 0.50 nm, 0.62 nm and 0.80 nm, respectively [22].

The cytokinin skeleton offers three regions for interaction with CDs: (1) substituent of amino group in position 6 of purine system; (2) purine nucleus; and (3) saccharide component of nucleosides.

In comparison to the saccharide part stronger interactions with hydrophobic cavity can be expected in the cases of the purine nucleus and substituent of amino group. The hydrophilic saccharide component can interact with the more polar outer surface of the CD molecule.

A typical course of the mobility dependence on diME-β-CD concentration can be seen in Fig. 1. Table 2 summarizes the differences in mobilities of studied compounds at 15 and 100 mmol l^{-1} concentration of each CD in comparison with BGE solution without CDs. Table 2 shows that all CDs strongly interact with free bases. This is probably caused by interaction between the CD and the purine ring, which is free for interaction when sugar is not bonded to the nitrogen in position 9.

α-CD strongly interacts with ''aliphatic'' cytokinins (especially with iP and iPR), kinetinriboside (KR) and with derivatives without sugar moiety (BAP, iP). This is caused by the interaction between the imidazole part of the purine system and α-CD.

β-CD derivatives have an important influence on the mobility of aromatic cytokinins (BAP, BAPR, BAPG, OTR, MTR) with benzenoid systems, which correspond to the β-CD cavity. The most intensive interaction was observed in the case of non-polar substituted diME-β-CD; the interaction with HP-β-CD and native β-CD is less intensive. A slightly higher interaction was observed for OTR than for MTR except for native β-CD. This is due to the sterically more advantageous position of the polar hydroxyl group in the *ortho* position in comparison to the *meta*-substituted derivative. Although β-CD and its derivatives also affect free bases, the influence is not as important as in the case of α- and γ-CDs.

γ-CD interacts first of all with the purine nucleus. That is why free bases are strongly retained by γ-CD. Weak discrimination was observed between ribosides and glucosides (BAPR and BAPG).

Fig. 2 shows the structures of individual substituents with their dimensions calculated from covalent radii of bonded atoms and valence angles. Approximate dimensions of the purine nucleus are 0.74×0.44 nm. The bigger proportion is in good agreement with the γ-CD cavity diameter, while the small imidazole nucleus (0.44 nm) is acceptable for the small cavity of α-CD. That is why both α- and γ-CDs can interact with free bases.

Dimensions of benzene or hydroxysubstituted benzene nucleus range from 0.51 to 0.57 nm according to the angle of observation. So it can be easily

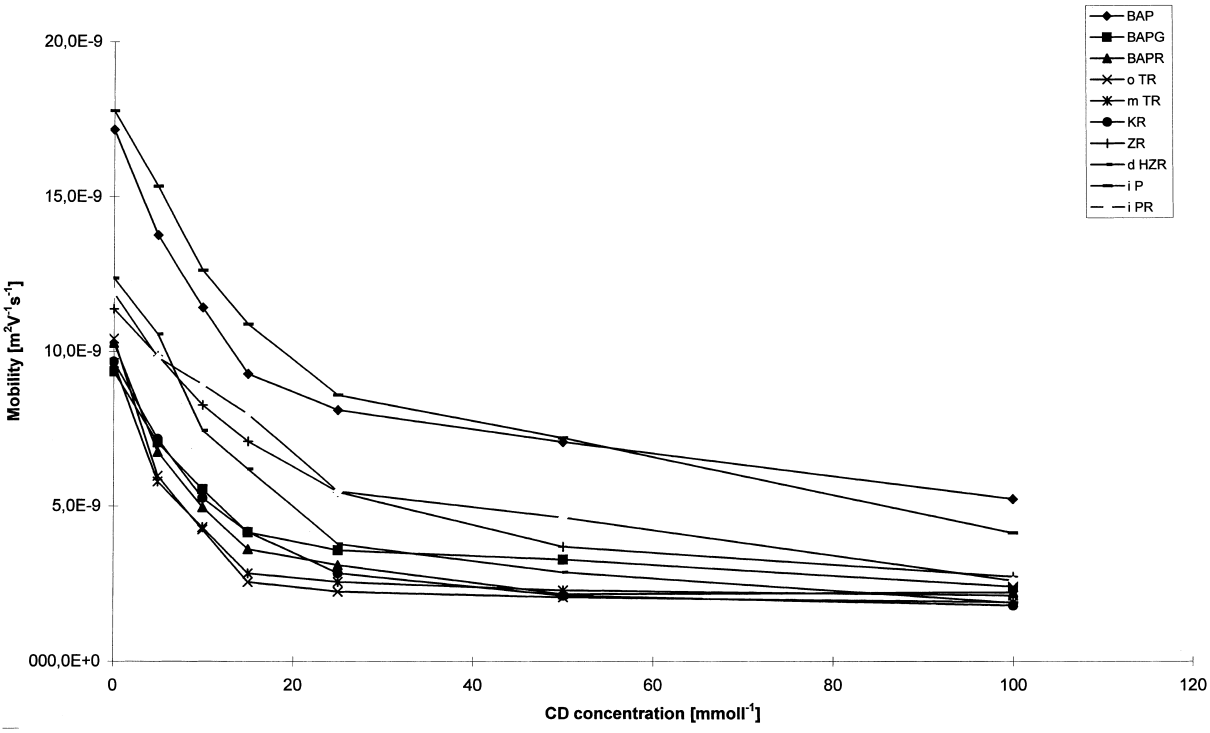


Fig. 1. Dependence of mobility of studied cytokinins on concentration of diME-β-CD.

incorporated into the β-CD and its derivatives cavity. Next, aromatic cytokinin – kinetin – is substituted by furan ring, which is substantially smaller (0.42 nm) than phenyl. So, the α-CD cavity is large enough for interaction with kinetin and its glycosides.

Observed strong interaction of aliphatic cytokinins with α-CD is difficult to explain. The contour of isopentenyl substituent is large enough (0.52 nm) for incorporation into the α-CD cavity, but only an inappreciable deformation of the CD ring and/or isopentenyl chain is sufficient for interaction.

Table 2
Differences in cytokinin mobilities in BGE without CD and with 15 or 100 mmol l^{-1} CD, respectively

	Mobility differences (mobility unit=10^{-9} m^2 V^{-1} s^{-1})								
	α-CD		β-CD	HP-β-CD		diMe-β-CD		γ-CD	
	0–15	0–100	0–15	0–15	0–100	0–15	0–100	0–15	0–100
BAP	4.09	8.43	6.98	7.00	10.67	8.41	11.94	2.25	8.99
BAPR	1.50	3.83	4.07	4.48	5.41	5.20	6.95	1.65	5.50
BAPG	1.84	4.43	4.93	5.26	6.19	6.66	8.06	1.85	6.37
OTR	1.32	3.88	4.48	5.67	8.04	7.86	8.51	2.07	6.52
MTR	1.02	3.25	4.67	5.04	7.09	6.79	7.51	2.02	6.01
KR	3.19	5.09	3.23	3.85	6.94	5.50	7.86	1.16	5.34
ZR	1.73	4.24	2.84	3.23	7.20	4.28	8.65	1.15	5.30
DHZR	1.28	4.83	3.94	4.43	10.46	6.18	10.49	1.92	5.73
IP	4.70	6.41	5.00	5.26	13.24	6.89	13.64	2.79	8.28
IPR	2.60	6.33	3.16	3.11	9.72	3.92	9.29	1.52	5.88

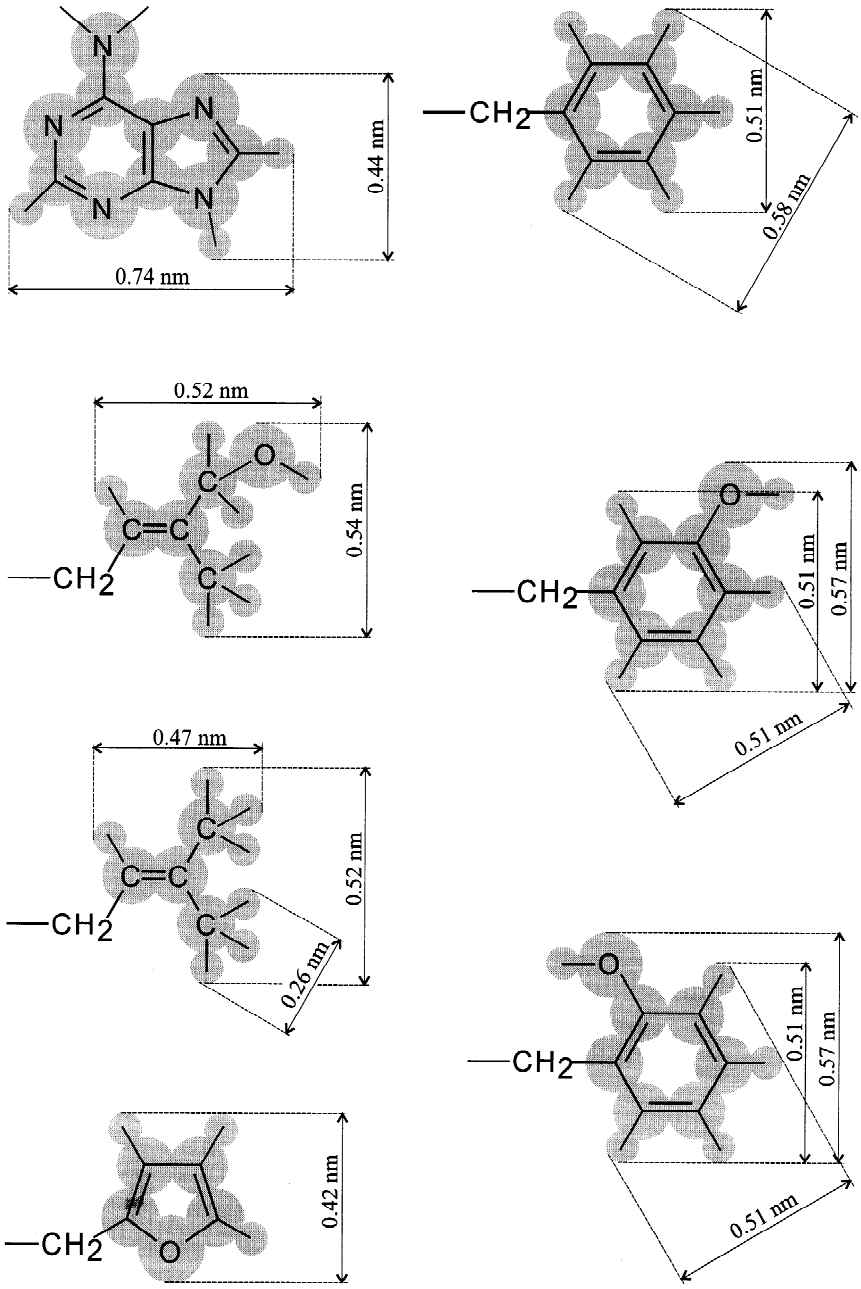


Fig. 2. Structures and approximate dimensions of cytokinins substituents.

Besides, ''transverse'' interaction with the isopentenyl chain is possible (0.47 nm). Interaction with some part of the isopentenyl substituent only (maybe one of the methyl groups – 0.26 nm) is another possibility. Weak interactions in the case of zeatin and dihydrozeatin support this hypothesis.

The formation of inclusion complexes was utilized for the full separation of a set of 10 cytokinins. Fig.

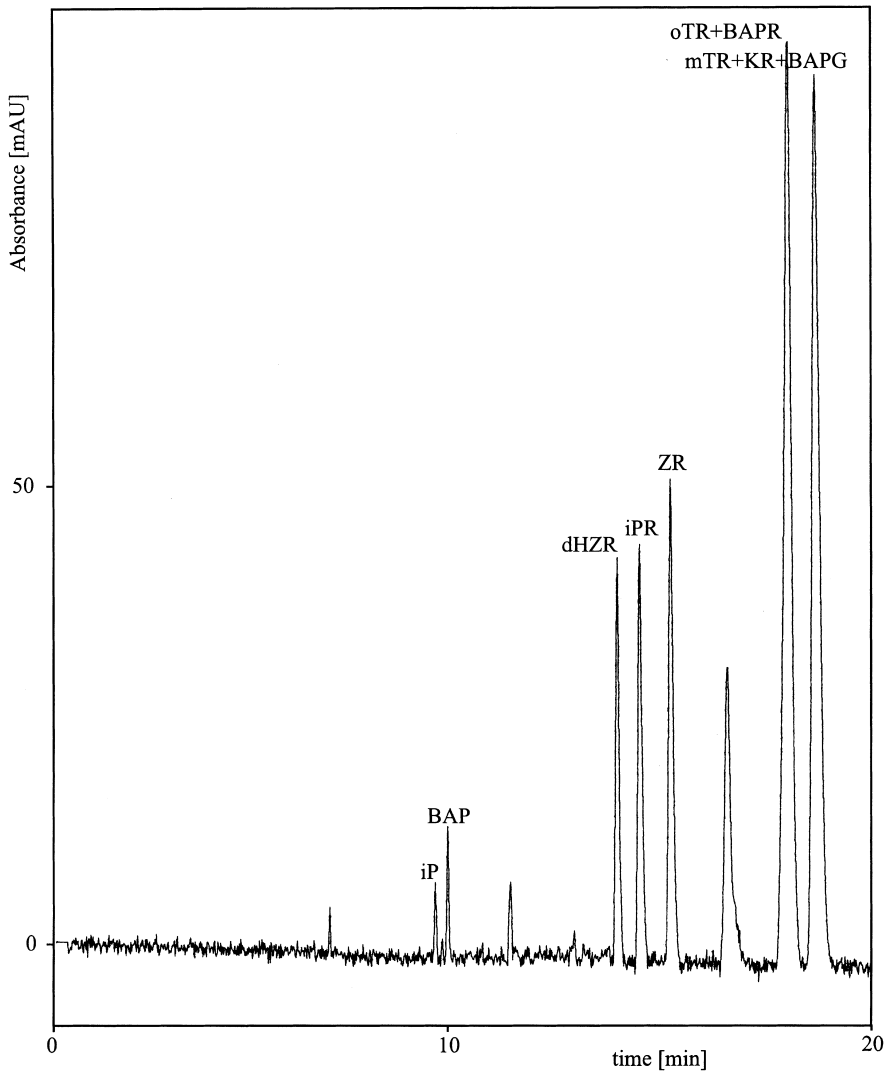


Fig. 3. Electropherogram of test mixture in 100 mmol l^{-1} phosphate–Tris (pH 2.5) buffer without CD (detection wavelength, 200 nm).

3 shows separation in BGE without CD addition. Full separation was reached with different types of CD at different concentrations. An electropherogram obtained with 25 mmol l^{-1} γ-CD in BGE is shown in Fig. 4 as an example of successful separation.

4. Conclusions

Interactions between cytokinins and different CDs were investigated. Each part of molecule contributes to the total interaction. Compounds without saccharide components interact especially with α- and γ-CDs. The substituent of amino group in position 6 of purine nucleus interacts first of all with α-CD (aliphatic substituents) and β-CD (aromatic substituents). Observed interactions are in correlation with the dimensions of a single part of molecule.

CE seems to be very good for the determination of a wide palette of purine derivatives. CE offers high efficiency and a very flexible separation system with

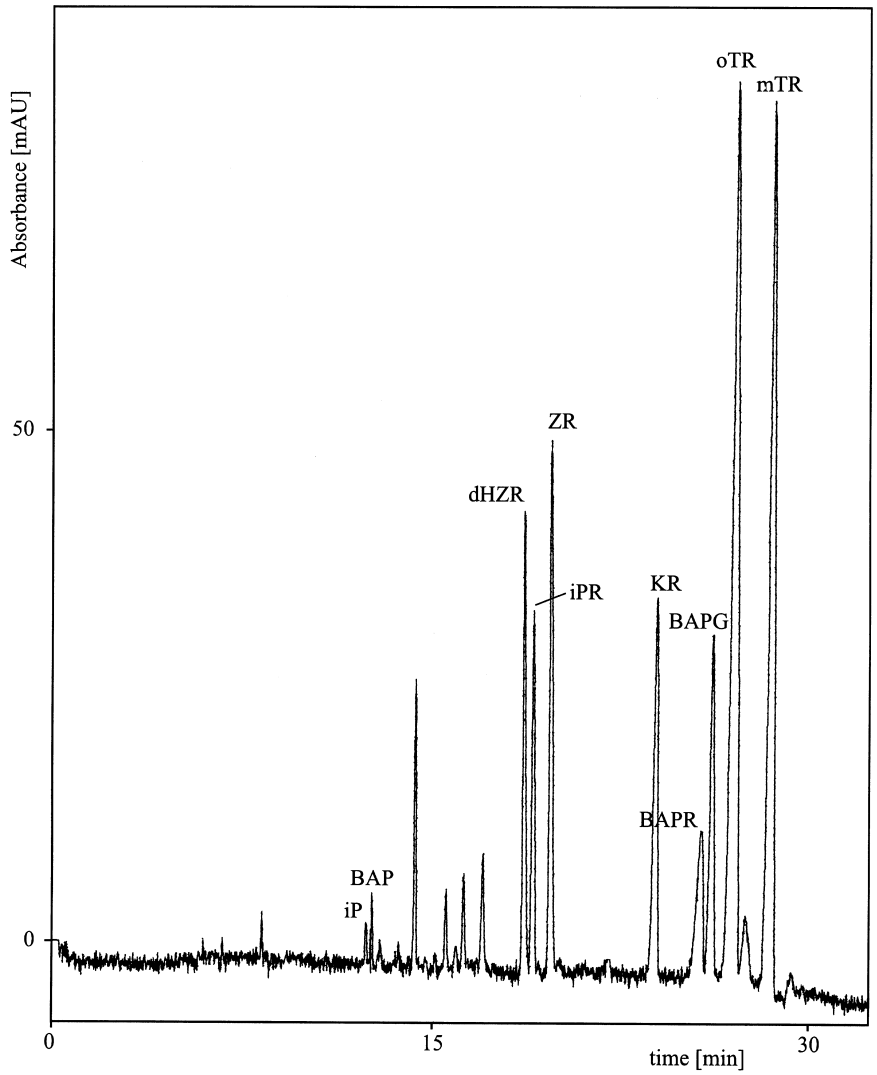


Fig. 4. Electropherogram of test mixture in 100 mmol l^{-1} phosphate–Tris (pH 2.5) buffer with 25 mmol l^{-1} γ-CD (detection wavelength, 200 nm).

sufficient sensitivity. It is much cheaper and faster than commonly used HPLC methods.

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