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Preparative separation of quinolines by centrifugal partition chromatography with gradient elution

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Abstract

Centrifugal partition chromatography has been successfully applied to the separation of 2-alkylquinolines from liquid combinatorial synthesis crude samples. Original gradient elution using the ternary two-phase solvent systems heptane–water–acetonitrile and heptane–acetonitrile–methanol were used to separate them with high purity degrees. Part of the effluent was monitored with evaporative light scattering detection, for direct control, and the collected fractions were analyzed by thin-layer chromatography, GC, nuclear magnetic resonance spectroscopy and MS. It was thus possible to purify in one run more than 3 g of crude mixture using only 1.3 l of solvents to obtain more than 300 mg of several alkylquinolines homologues with 99% purity and in less than 7 h.

Keywords: Preparative chromatography; Centrifugal partition chromatography; Gradient elution; Combinatorial chemistry; Alkaloids; Quinolines

1. Introduction

Leishmaniasis is generally found in tropical and subtropical parts of the world and is caused by infection with protozoan of the genus *Leishmania* transmitted by the bites of infected fly. Leishmaniasis is considered to be endemic in 88 countries on five continents and the World Health Organization (WHO) estimates an incidence of 12 million cases among 350 million at risk and an annual incidence of 1.5 million new cases [1]. Leishmaniasis can be divided into three major groups on the basis of the tissue tropisms of the parasite and the clinical manifestations: first, those which are restricted to the skin causing cutaneous leishmaniasis with lesions mainly on the face, arms and legs and creating serious disability and permanent scars; secondly, mucocutaneous leishmaniasis causing disfiguring lesions to the face; and finally those which infect internal organs such as liver, spleen, bone marrow and cause visceral leishmaniasis usually fatal if left untreated [2].

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Cutaneous and visceral leishmaniasis are common infections in the subtropical areas of the Department of La Paz (Bolivia). The medicinal treatments (pentavalent antimony, amphotericin B, miltefosine) are too expensive or unavailable for the population suffering from leishmaniasis. The use of medicinal plants is commonplace, especially among the Chimane Indians, a group that inhabits the gallery forest. Among these plants, a tree is used for the treatment of cutaneous leishmaniasis. The stem bark, in the form of poultice, is applied on the cutaneous leishmaniasis until complete cicatrisation of the wound. This tree, growing in a specific area of the Amazonian forest located in the North East of the Bolivia, was botanically identified as *Galipea longiflora* from the Rutaceae family. The activity-guided purification of the stem barks, roots barks and leaves extracts of this plant afforded several active compounds identified as 2-alkyl- and 2-alkenylquinolines called chimanines [3]. They have shown very promising activity against several *Leishmania* strains, and in vivo tests have demonstrated their oral leishmanicide property [4,5]. Some activity against *Plasmodium vinckei petteri* was also expressed by some of them [6]. Therefore, in our structure–activity relationships studies, we were interested in the separation of homologue quinolines, possessing different alkyl chains branched at the 2-position of the quinoline nucleus. The most active compounds against leishmania strains being the 2-propyl, 2-propenyl and 2-pentylquinolines, the influence of the alkyl chain length on their activity was screened.

Instead of a separate mode of synthesis for each alkaloid with its own purification process, we preferred a liquid combinatorial synthesis approach, followed by one-step purification of all the compounds using centrifugal partition chromatography (CPC).

CPC is a preparative liquid–liquid chromatographic technique, without any solid support, based on partition of solutes between two immiscible liquid phases [7–10]. One phase is retained as stationary phase in the column partition channels by the action of a constant centrifugal force while the other phase, the mobile phase, flows continuously through the stationary phase. Compounds are separated according to their distribution constants K_c expressed as the ratio of their concentration in the stationary phase to their concentration in the mobile phase.

This paper describes the selection of solvent systems based on solubility, partition coefficient studies and ternary diagram analysis, as well as the preparative separation of 2-alkylquinolines from liquid-phase combinatorial synthesis crude samples, using original gradient elutions.

2. Experimental

2.1. Preparation of the quinoline mixtures

Quinoline mixtures were prepared according to the previously detailed liquid combinatorial synthesis approach [11]. The mixtures of 2-alkylquinolines were obtained in a single pot adding directly a mixture of *n*-alkyl Grignard reagents on the *N*-oxycarbonylisobutyloxyquinolinium chloride (Fig. 1).

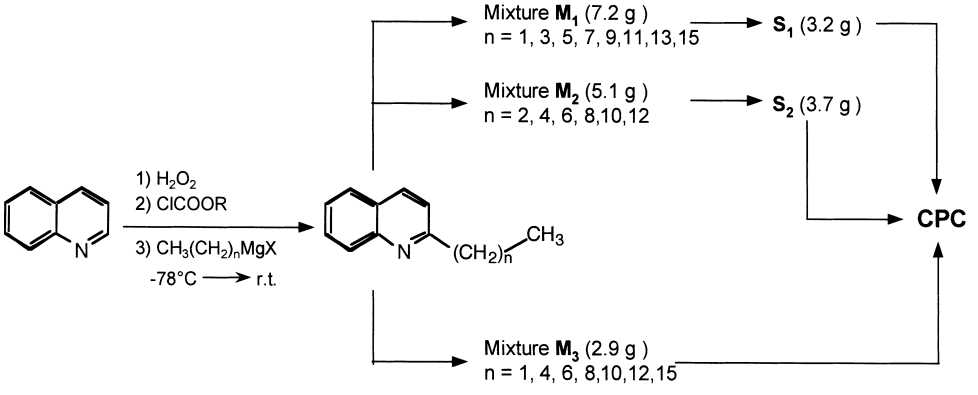

Fig. 1. Liquid combinatorial synthesis of the 2-alkylquinolines [11].

Three mixtures were prepared: the first one M_1 was a mixture of eight 2-alkylquinolines ($n=1$, 3, 5, 7, 9, 11, 13, 15), the second M_2 was a mixture of six 2-alkylquinolines ($n=2$, 4, 6, 8, 10, 12) and the third M_3 was a mixture of seven 2-alkylquinolines ($n=1$, 4, 6, 8, 10, 12, 15). Preliminary separations of the quinolines and the by-products, such as residual *N*-oxyquinoline and alkanes corresponding to the hydrolyzed Grignard reagents, were performed before experiments on the two mixtures M_1 (7.2 g) and M_2 (5.1 g). Thus, the two mixtures were treated in a similar manner: after filtration, the crude samples were dissolved in the upper organic phase of the biphasic heptane–methanol–water (4:3:1, v/v) system. Three successive extractions with 95% H_2SO_4 acidified aqueous lower phase (3:100, v/v) were performed, in order to extract the 2-alkylquinolines in their cationic forms by the acidic aqueous phases. The acidic aqueous phases were then alkalinized with a 20% NH_4OH solution until pH 9 and then the 2-alkylquinolines extracted four times in their basic forms with the upper organic phase of the heptane–methanol–water (4:3:1, v/v) system. The organic phases were washed, dried, filtered and carefully evaporated under reduced pressure to yield yellow oils S_1 (3.2 g) and S_2 (3.7 g) issued from the mixtures M_1 and M_2, respectively. The third mixture M_3 (2.9 g) was directly injected without any preliminary treatment (Fig. 1).

2.2. CPC apparatus

For all experiments, an LLB high-performance centrifugal partition chromatography (HPCPC) system (Sanki, Kyoto, Japan) was used. The total column volume is 220 ml. This HPCPC system was equipped with a quaternary solvent delivery pump Waters Model 600 (Waters, Milford, MA, USA). A flow splitter was installed at the outlet of the HPCPC—the main line (90% of the flow) going to a fraction collector, ISCO type Retriever 500 (ISCO, Lincoln, NB, USA)—the other line (10% of the flow) going to an evaporative light scattering detection (ELSD) system, Sedere type Sedex 45 (Sedere, Vitry-sur-Seine, France) [12,13]. The temperature and pressure of the nebuliser were set at 30 °C and 2.2 bar, respectively. The samples were injected through a 7725i Rheodyne valve with a 15-ml sample loop made in the laboratory (Rheodyne, Cotati, CA, USA).

2.3. Reagents

For CPC separations, distillated water, commercial analytical-grade heptane and methanol from Prolabo (Paris, France), and HPLC-grade acetonitrile from Carlo Erba (Val de Rueil, France) were used. Heptane and methanol were distillated before use.

2.4. Measurement of the distribution constants K_c and control of the collected CPC fractions

Shake flask experiments were performed to determine the distribution constants K_c of the 2-alkylquinolines between the two phases of the biphasic tested systems [7,14]. Various two-phase systems were prepared by stirring at room temperature heptane, water, acetonitrile and methanol with different volume ratios before separating into upper and lower phases. To measure the distribution constants, an aliquot (1–5 mg) of the mixtures S_1, S_2 or M_3 was dissolved in 1–5 ml of the upper (or lower) phase and the solution was gently shaken with an equal volume of lower (or upper) phase for 10 min. The two phases were then separated and the solute concentrations in each phase were first estimated by thin-layer chromatography (TLC) and then quantified by gas chromatography (GC). For each solute the distribution constant K_c was calculated as follows:

$$K_c = [C]_{upper}/[C]_{lower} = A_{upper}/A_{lower} \quad (1)$$

where $[C]_{upper}$ and $[C]_{lower}$ are the solute concentrations in the upper and lower phases, respectively; A_{upper} and A_{lower} are the GC solutes peak areas in the upper and lower phases, respectively.

Each collected fraction obtained by CPC was analyzed by TLC on silica gel $60F_{254}$ plates (Merck, Darmstadt, Germany): the mobile phase was a toluene–ethyl acetate (85:15, v/v) mixture; spots were visualized under UV light (254 and 365 nm) and by Dragendorff reagent. Identical fractions were pooled and carefully evaporated to dryness. These pooled fractions, showing one spot by TLC, were then controlled by GC and ^{1}H-nuclear magnetic

resonance (NMR) spectroscopy on a Bruker AC-200 machine at 200 MHz (C^2HCl_3) [11].

A H 5890A gas chromatograph [Hewlett-Packard (HP), Evry, France] equipped with a flame ionization detector, a HP 7673 injector and a HT5 SGE capillary column (25 m × 0.22 mm I.D.) coated with 0.1 μm 5% phenylmethylpolysiloxane (SGE, Villeneuve-Saint-Georges, France), was used for the analysis. The injector and detector temperatures were 300 and 350 °C, respectively, and nitrogen was used as carrier gas at a flow-rate of 0.7 ml/min (split 100 ml/min, purge 4 ml/min). A HP 3392A recorder integrator was used for the measurement of retention times and peak area determinations. The column temperature was programmed from 100 to 360 °C at 5 °C/min and held at 360 °C during 10 min.

2.5. CPC separation procedure

2.5.1. Separation of quinolines from S_1 and S_2

To separate the substituted quinolines from these two mixtures we selected a two-phase solvent system composed of heptane–acetonitrile–methanol with various volume ratios and a gradient elution [7,15,16].

The initial mobile phase (acetonitrile saturated with heptane), the final mobile phase (methanol saturated with heptane) and the stationary phase (heptane saturated with acetonitrile) were prepared separately by mixing the solvents until a biphasism occurred. Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and filtered shortly before use on a Millipore 0.45 μm HVHP type filter (Millipore, Bedford, MA, USA).

The HPCPC column was first entirely filled with the stationary phase, and then the apparatus was rotated at 1000 rpm while the initial mobile phase was pumped through the column in descending mode at 2–3 ml/min. After the mobile phase front emerged and equilibrium was established in the column (72% retention of the stationary phase), 15 ml of the sample solutions (S_1 or S_2 dissolved in a mixture of stationary and initial mobile phases in v/v ratio), were injected with the Rheodyne injection valve at a flow-rate of 0.5 ml/min. The flow-rate was slowly increased until the operational flow-rate was reached (2–3 ml/min). Elution remained 90 min isocratic with the initial mobile phase (acetonitrile saturated with heptane) before linear gradient to 100% final mobile phase (methanol saturated with heptane) was established in 180–240 min. Then isocratic elution was performed with the final mobile phase until elution of the last compound was observed. The drop pressure was 30 to 15 bar. The effluent of the column was continuously monitored with ELSD and the collected fractions (3–5 ml) were analyzed by TLC and GC. At the end of each run, methanol was pumped through the column at 10 ml/min with a 200 rpm rotor speed to push the stationary phase and to wash the column.

2.5.2. Separation of quinolines from M_3

A double gradient elution, involving two two-phase solvent systems, was used in the case of M_3. A first gradient elution was performed with the upper and lower phases of the heptane–acetonitrile–water (5:1:4, v/v) two-phase system used as stationary and initial mobile phases, respectively. A linear variation of the lower phase composition was performed from this initial mobile phase IMP1 to the final mobile phase of the first system FMP1 (lower phase of heptane–acetonitrile) which corresponds to the initial mobile phase IMP2 (acetonitrile saturated with heptane) of the second two-phase system: heptane–acetonitrile–methanol. Then, the same gradient elution system than in the case of S_1 and S_2, was used from heptane–acetonitrile to heptane–methanol.

The column was first entirely filled with the upper phase of the heptane–acetonitrile–water (5:1:4, v/v) two-phase system used as stationary phase, then the apparatus was rotated at 1200 rpm while the lower phase of this two-phase system, used as initial mobile phase IMP1, was pumped through the column in descending mode at a flow-rate of 2.5 ml/min. With these conditions the volume of the mobile phase in the CPC column was 40 ml, corresponding to retention of the stationary phase of 82%. The M_3 sample (2.9 g), dissolved in 15 ml mixture of stationary and mobile phases in v/v ratio, was injected with the Rheodyne injection valve at the flow-rate of 0.5 ml/min, then the flow-rate was slowly increased to 2.5 ml/min. An isocratic elution with the initial mobile phase IMP1 was performed during 30 min before running a linear gradient to 100% final mobile phase FMP1 (lower phase of the

heptane–acetonitrile two-phase system) in 30 min. Next, elution remained 60 min isocratic before running a second linear gradient to 100% final mobile phase FMP2 (lower phase of the heptane–methanol system) in 240 min. Finally, isocratic elution with the final mobile phase FMP2 was performed during 100 min. The back-pressure was 40 to 15 bar. Detection was continuously performed with ELSD and the collected fractions (7.5 ml) were analyzed by TLC and GC. After the last fraction was collected, methanol was pumped through the column, to push out the stationary phase and wash the column.

3. Results and discussion

Organic reactions of large scope performed in solution remain an attractive tool for the access to a large number of bioactive compounds. The ideal process would be thus to synthesize in solution a large number of compounds in one pot, then either to perform the biological tests on the mixtures or to separate all constituents for individual screenings. In order to obtain a library of 2-alkylquinolines readily accessible for biological tests, three mixtures S_1, S_2 and M_3 were prepared. TLC of the crude mixtures showed in any case broad spots, hardly separable by conventional flash chromatography. Preparative high-performance liquid chromatography (HPLC) analyses of these mixtures would have required large quantities of solvents, as well as the desired columns and equipment. Therefore we turned our attention to the separation by CPC which combined an inexpensive procedure (few solvent without expensive columns) and a very efficient separation process when the chromatographic conditions are well optimized.

The preliminary step was the two-phase solvent system selection. The first thing to do was to find the solvents where the samples S_1, S_2 and M_3 were most soluble. Various solvents covering a wide range of polarity, including water, methanol, acetonitrile, acetone, ethyl acetate, *tert.*-butyl methyl ether, dichloromethane, *n*-hexane, *n*-heptane have been tested.

Acetonitrile, methanol, dichloromethane, hexane and heptane were selected as the best solvents for the three mixtures S_1, S_2 and M_3. The solubility of the samples in these solvents, which have different polarity index, could be explained as the result of the additional solubilities of all the compounds present in the mixtures and which differ by their alkyl chain length.

Dichloromethane and hexane were not kept because of their toxicity and the heptane–acetonitrile–methanol and heptane–acetonitrile–water systems were studied in first intention with different ratios (Table 1). The partition coefficients of the 2-alkylquinolines between the two phases of the biphasic tested systems were first estimated with the shake-flask method coupled with TLC and finally quantified using GC.

The distribution coefficients values determined by GC and obtained for the 2-alkylquinolines with the six two-phase tested systems are compiled in Table 1. When water is present in the two-phase system (heptane–acetonitrile–water, 5:3:2 or 5:4:1, v/v) the obtained distribution coefficients show greater solubility of the compounds in the upper organic phase. In the heptane–acetonitrile biphasic system the 2-alkylquinolines with short alkyl chain show K_c values close to 1 (from 0.56 for the 2-ethylquinoline to 1.31 for the 2-pentylquinoline) whereas the compounds with longer carbon chain stay preferentially in the upper heptane phase (K_c from 1.76 for the 2-hexylquinoline to 35.55 for the 2-hexadecylquinoline).

Experimental partition coefficient data for 2-alkylquinolines as a function of the carbon chain length (n) show a linear relationship between $\ln K_c$ and n for each heptane–acetonitrile–methanol biphasic tested system (Fig. 2) and the partition coefficients were fitted with the exponential relationship:

$$K_c = a\mathrm{e}^{bn} \Rightarrow \ln K_c = bn + \ln a \quad (2)$$

where a and b are constants for each biphasic system. These constants are given in Table 2 and Fig. 2 clearly shows that the previous equation gives a good representation of the data. As in reversed-phase HPLC [17–19], this empirical relationship might be valid for homologous series of compounds, allowing one to predict K_c values or to identify compounds in a homologous series. We can also observe that, the more methanol is added, the more

Table 1
Distribution constants[a] (K_c) of the 2-alkylquinolines (C_n-Q) in various solvent systems

Carbon chain	Heptane–acetonitrile–water (v/v)			Heptane–acetonitrile–methanol (v/v)		
	(5:3:2)	(5:4:1)	(5:5:0)	(5:3:2)	(5:1:4)	(5:0:5)
C_2	nd[b]	nd	0.56	0.33	0.32	0.32
C_3	nd	nd	0.77	0.40	0.37	0.37
C_4	nd	nd	1.04	0.53	0.46	0.44
C_5	18.57	2.59	1.31	0.66	0.55	0.51
C_6	nd	nd	1.76	0.87	0.68	0.59
C_7	22.88	5.55	2.36	1.11	0.82	0.68
C_8	nd	nd	3.18	1.42	0.99	0.78
C_9	nm[c]	11.94	4.21	1.83	1.20	0.88
C_{10}	nd	nd	5.54	2.33	1.44	1.02
C_{11}	nm	24.63	7.24	3.14	1.73	1.26
C_{12}	nd	nd	10.21	3.81	2.04	1.42
C_{13}	nm	26.30	12.92	4.73	2.46	1.60
C_{14}	nd	nd	17.59	6.26	2.98	1.79
C_{16}	nm	nm	35.55	10.22	4.25	2.37

[a] Partition coefficients (K_c) of solutes were measured at 20 °C with the ''shake-flask'' method coupled with GC quantitative analyses, and K_c values were calculated as the solute affinity for the stationary phase: $K_c = [C]_{\text{upper phase}} / [C]_{\text{lower phase}}$ (1)
[b] Non determined.
[c] Non measurable (no solute was detected by GC in the lower aqueous phases tested).

the 2-alkylquinolines are displaced to the methanolic lower phase.

Moreover, the experimental partition coefficient data show that the K_c values of each component i depend on the methanol content X of the heptane–acetonitrile–methanol systems according to:

$$K_{ci} = A_i e^{B_i X} \quad (3)$$

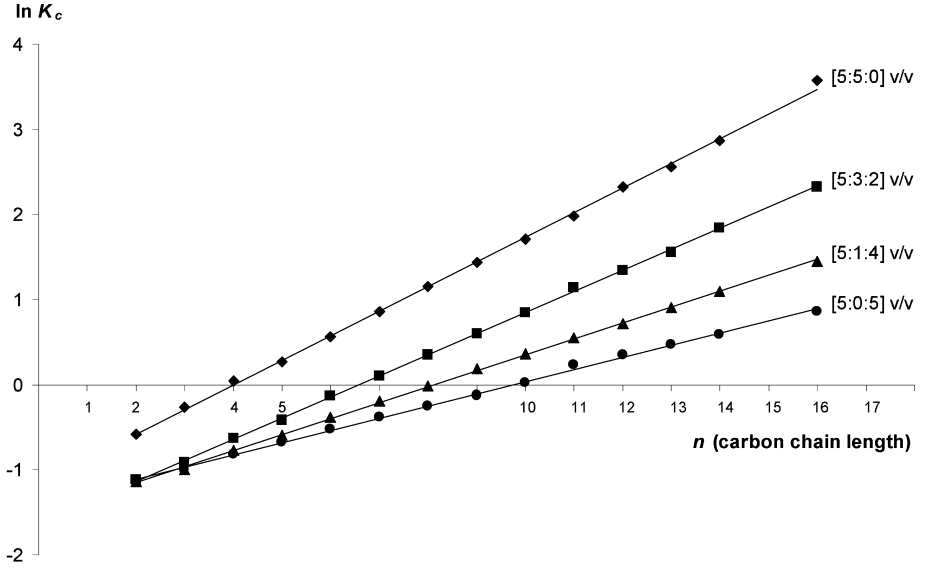

Fig. 2. Partition coefficients of 2-alkylquinolines with different chain lengths with heptane–acetonitrile–methanol biphasic system. Markers are experimental data, lines are fitted with Eq. (2).

Table 2
Linear relation between ln K_c and the alkyl chain length (n) (Eq. (2))

Biphasic solvent system, heptane–acetonitrile–methanol (v/v)	$K_c = ae^{bn} \Rightarrow \ln K_c = bn + \ln a$		
	b	Ln a	Correlation (R)
(5:5:0)	0.290	−1.161	0.9991
(5:3:2)	0.247	−1.628	0.9996
(5:1:4)	0.187	−1.518	0.9996
(5:0:5)	0.144	−1.403	0.9985

where A_i and B_i (given in Table 3) are constants for component i and X is the volumetric methanol content [20]. This relationship is closely related to the retention factor equation (k'), obtained in reversed-phase HPLC, as the function of the organic solvent percentage in the mobile phase ($k' = Ae^{BX}$, with X the % of the organic solvent) [21–23]. This shows that this two-phase system with heptane as stationary phase can be approximated to reversed-phase chromatography.

It is shown in Fig. 3 that no efficient separation of all the quinolines would be possible with one of these systems in isocratic elution mode because of no good compromise between high selectivities and sufficiently different K_c values.

This problem was solved by the analysis of the ternary phase diagram of the heptane–acetonitrile–methanol system. Fortunately, this diagram was described in the literature by Foucault [7].

Orthogonal representation of this diagram is shown in Fig. 4. It describes how methanol, acetonitrile and heptane mix together. Tie-line orientation clearly indicates that methanol prefers to go into acetonitrile than into heptane. Considering the A, B, C and D systems, we can see, by following their tie-lines, that the respective upper non polar phases are identical while the respective lower polar phases differ by the methanol content. Because of the converging tie-lines, this system is favorable for a gradient run in the reverse phase mode [7,15,16]. All the tie-lines converge to the SP (stationary phase) point corresponding to the composition of the upper stationary phase which is identical for all the biphasic mixtures and immiscible with the corre-

Table 3
Coefficients A_i and B_i for the 2-alkylquinolines (Eq. (3))

Carbon chain length C_n-quinoline	$K_{ci} = A_i e^{B_i X}$		Experimental K_c obtained with heptane–CH_3CN–CH_3OH (5:5:0, v/v)
	A_i*	B_i	
C_2	0.50	−0.106	0.56
C_3	0.67	−0.141	0.77
C_4	0.91	−0.166	1.04
C_5	1.16	−0.183	1.31
C_6	1.58	−0.211	1.76
C_7	2.13	−0.241	2.36
C_8	2.89	−0.272	3.18
C_9	3.88	−0.303	4.21
C_{10}	5.15	−0.328	5.54
C_{11}	6.86	−0.346	7.24
C_{12}	9.45	−0.387	10.21
C_{13}	12.10	−0.408	12.92
C_{14}	16.81	−0.447	17.59
C_{16}	33.38	−0.530	35.55

*X is the volumetric methanol content (with $X > 0$). When $X \to 0$; $K_c \to A_i$

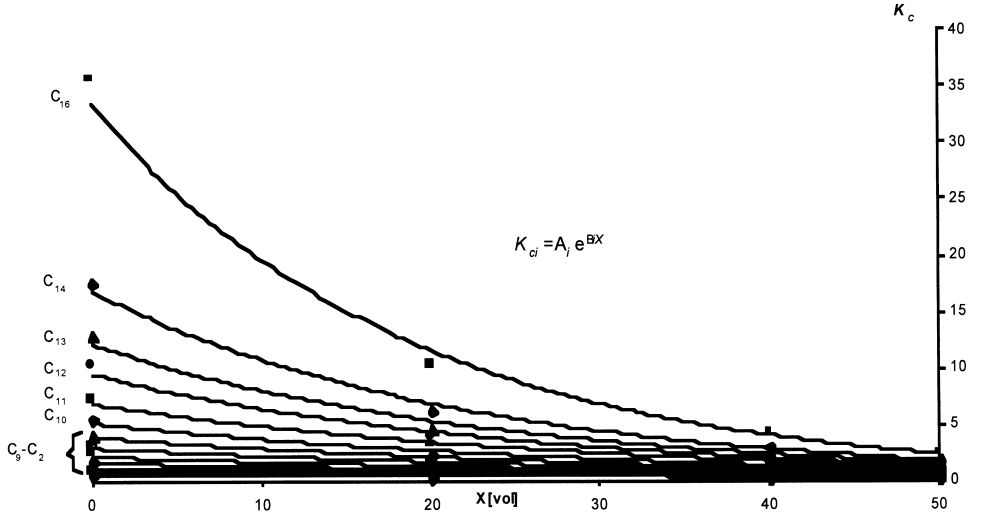

Fig. 3. K_c as a function of X (vol of MeOH). Markers are experimental data, lines are fitted with Eq. (3).

sponding lower phases. If the CPC instrument is filled with this heptane-rich stationary phase SP, it would be possible to vary the solvent-strength of the lower mobile phase by increasing the methanol volume percentage from 0 to 70%. Gradient elution should be performed by linearly varying the composition of the lower phase from the initial mobile phase (IMP) point which corresponds to the lower phase of system A (acetonitrile saturated with hep-

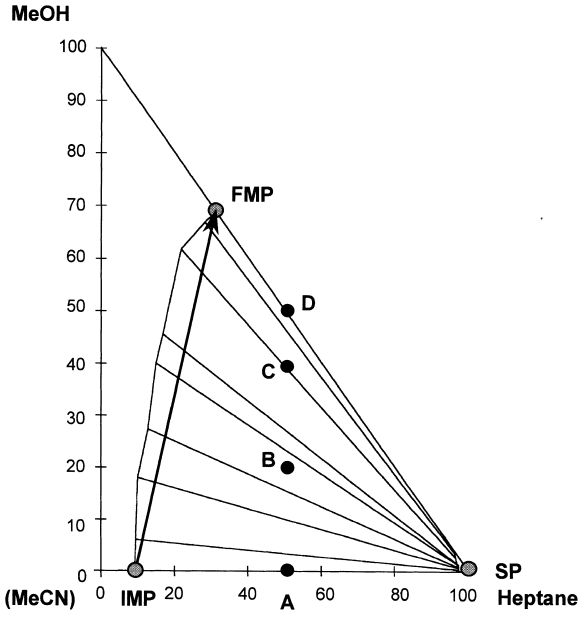

Fig. 4. Orthogonal representation of the tertiary phase diagram of the heptane–acetonitrile–methanol system [7]. SP = Stationary phase, IMP = initial mobile phase, FMP = final mobile phase.

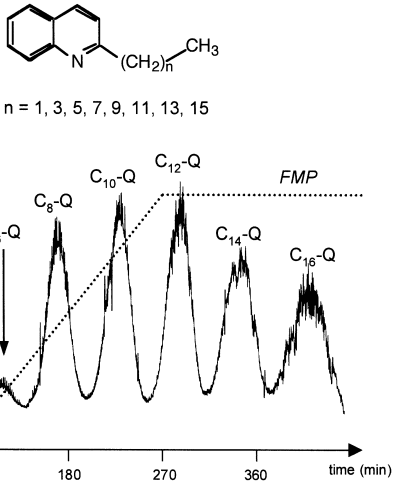

Fig. 5. CPC–ELSD chromatogram of the mixture S_1 of eight 2-alkylquinoline (from the 2-ethylquinoline C_2-Q to the 2-hexadecylquinoline C_{16}-Q). Stationary phase: heptane saturated with acetonitrile, initial mobile phase: acetonitrile saturated with heptane (isocratic mode during 90 min), final mobile phase: methanol saturated with heptane (180 min linear gradient from IMP to FMP), rotation speed: 1000 rpm; flow rate: 3 ml/min in descending mode, ELSD nebulizer temperature: 30°C; ELSD nebulizer air pressure: 2.2 bar (for the detailed chromatographic conditions see the *CPC separation procedure* part in the Experimental section).

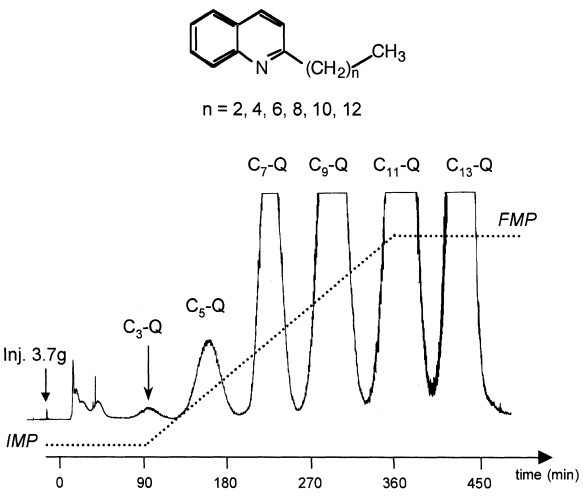

Fig. 6. CPC–ELSD chromatogram of the mixture S_2 of six 2-alkylquinoline (from the 2-propylquinoline C3-Q to the 2-tridecylquinoline C13-Q). Stationary phase: heptane saturated with acetonitrile, initial mobile phase: acetonitrile saturated with heptane (isocratic mode during 90 min), final mobile phase: methanol saturated with heptane (240 min linear gradient from IMP to FMP), rotation speed: 1000 rpm; flow rate: 2 ml/min in descending mode, ELSD nebulizer temperature: 30°C; ELSD nebulizer air pressure: 2.2 bar (for the detailed chromatographic conditions see the *CPC separation procedure* part in the Experimental section).

tane), to the final mobile phase (FMP) point which corresponds to the lower phase of system D (methanol saturated with heptane).

This linear gradient was tested to separate the substituted quinolines from the two samples S_1 and S_2. The chromatograms obtained after injections of 3.2 and 3.7 g of the mixtures S_1 and S_2 are presented in Figs. 5 and 6, respectively.

All collected fractions were checked by TLC and the identical fractions were pooled and carefully evaporated to dryness. Pooled fractions containing one compound (one spot in TLC) were finally controlled by GC, mass spectrometry and ^{1}H-NMR spectra analysis. The elution was similar to that in octadecylsilyl reversed-phase liquid chromatography and the quinolines were eluted according to the length of their alkyl chain. The total separation time was around 7 h with a total elution volume of around 1.2 l. Low detector signals and yields (recovering) may be due to the volatility of the quinolines in the oven of the ELSD system, and during the evaporation of the collected fractions.

All the quinolines were obtained in one step with a chromatographic purity from 90 to 99% except for the 2-ethylquinoline, which was obtained with 60%

Table 4
Alkylquinolines isolated from S_1 and S_2 mixtures

2-Alkylquinolines C_n-Q	Amount isolated from S_1 (mg)[a]	Amount isolated from S_2 (mg)[a]	GC purity (%)
C_2	80	–	60[b]
C_3	–	60	90
C_4	110	–	90
C_5	–	168	99
C_6	179	–	90
C_7	–	230	>99
C_8	207	–	99
C_9	–	257	>99
C_{10}	236	–	99
C_{11}	–	401	>99
C_{12}	264	–	99
C_{13}	–	382	>99
C_{14}	394	–	>99
C_{16}	328	–	>99
Total (mg)	1798	1498	
Yield (%)	56	40	

[a] The compiled values do not take into account the lost of compounds, due to the volatility of the quinolines, which occurred during the evaporation of the collected fractions, and the lost due to the 10% effluent going to the ELSD system.

[b] Obtained in mixture with residual *N*-oxyquinoline.

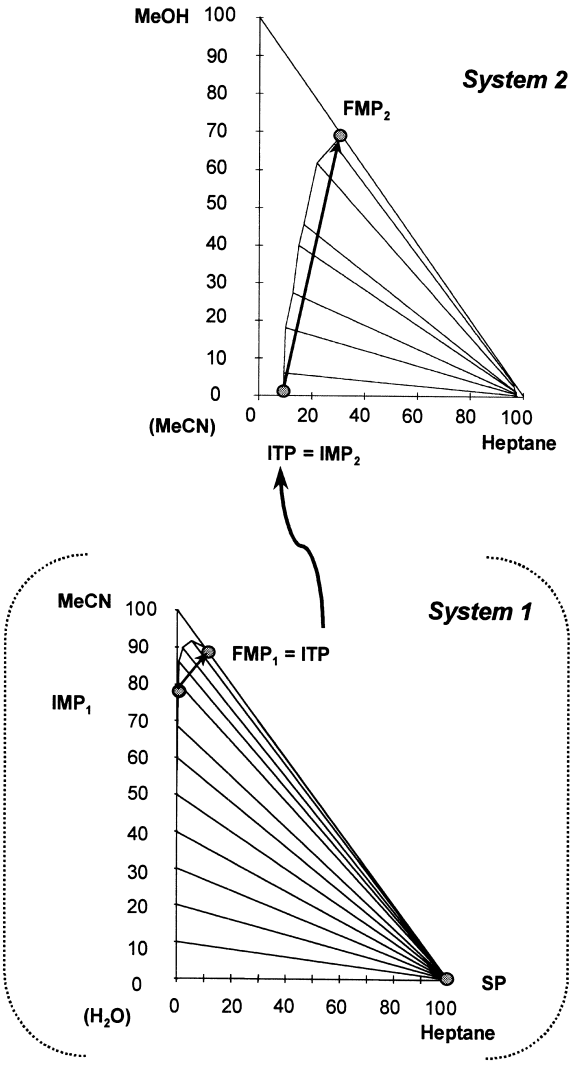

Fig. 7. Double gradient elution. *System 1:* **virtual** orthogonal representation of the ternary phase diagram of heptane–water–acetonitrile system. **This diagram was supposed to be very close to the diagram of the system heptane–water–methanol** [7]. SP = Stationary phase (upper phase of heptane–water–acetonitrile, 5:1:4, v/v); IMP_1 = initial mobile phase (lower phase of heptane–water–acetonitrile, 5:1:4, v/v); FMP_1 = final mobile phase of the first gradient system (lower phase of heptane acetonitrile, 5:5, v/v) corresponding to the ITP = intermediate mobile hase. *System 2:* orthogonal representation of the ternary phase diagram of the heptane–acetonitrile–methanol system [7]. IMP_2 = initial mobile phase (lower phase of heptane–acetonitrile) corresponding to the intermediate mobile phase ITP; FMP_2 = final mobile phase (methanol saturated with heptane).

purity mixed with residual *N*-oxyquinoline. The results are summarized in Table 4.

The observed elution sequence being in conformity with the reversed-phase elution, the separation of the *N*-oxyquinoline/2-ethylquinoline mixture was performed by adding water in the mobile phase to reduce the solvent-strength of the mobile phase and to enhance the separation of these two compounds. So a double gradient elution was envisaged. This double gradient elution resulted from the combination of two biphasic systems. The first one was heptane–acetonitrile–water, a ternary phase diagram which has not been yet reported in the literature. Nevertheless it was supposed to be very close to the heptane–methanol–water system for which it is possible to vary the lower phase composition without modification of the upper phase (Fig. 7) [7]. So a double gradient elution could be performed by varying the mobile phase composition from the initial aqueous mobile phase IMP1 of the first system to the final methanolic mobile phase FMP2 of the second system dropping by an intermediate phase ITP (FMP1 or IMP2) which is common to the two systems (Fig. 7). This original approach was applied to separate the seven quinolines of the mixture M_3 using this double gradient elution. As shown in Fig. 8, all the compounds were separated in one step and the 2-ethylquinoline was obtained pure without any residual *N*-oxyquinoline.

4. Conclusion

Fourteen different 2-alkylquinolines have been isolated in one step from three crude mixtures issued from liquid phase combinatorial synthesis and were obtained with high purity in sufficient amount for in vivo biological evaluations. Original single and double gradient elutions in the reverse mode elution were applied with success to separate all these compounds which were eluted, as in reversed-phase HPLC [17–19], according to the length of their alkyl chain. This suggests that these gradients could be used to a broad variety of homologous series of compounds without the disadvantages of the classical reversed-phase HPLC such as the poor column capacity, the important solvent consumption and the solid stationary phase cost.

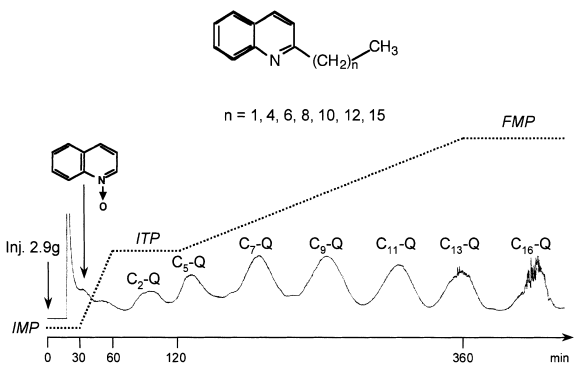

Fig. 8. CPC–ELSD chromatogram to the mixture M_3 of seven 2-alkylquinoline (from the 2-ethylquinoline C2-Q to the 2-hexadecylquinoline C16-Q). Stationary phase: upper phase of heptane–water–acetonitrile (5:1:4 v/v), initial mobile phase (IMP): lower phase of heptane–water–acetonitrile (5:1:4, v/v) (isocratic mode during 30 min), intermediate mobile phase (ITP): acetonitrile saturated with heptane (isocratic mode during 60 min), final mobile phase (FMP): methanol saturated with heptane (240 min linear gradient from ITP to FMP), rotation speed: 1200 rpm; flow rate: 2.5 ml/min in descending mode, initial volume of stationary phase: 180 ml (82% retention), final volume of stationary phase: 132 ml (20% leakage), ELSD nebulizer temperature: 30°C; ELSD nebulizer air pressure: 2.1 bar (for the detailed chromatographic conditions see the *CPC separation procedure* part in the Experimental section).

Moreover, this double gradient elution from aqueous mobile phase to methanolic mobile phase could be used for a rapid fractionation of crude extracts especially included in a high-throughput screening (HTS) strategy for new lead discovery.

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