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DERIVATIZATION OF SECONDARY ALIPHATIC ALCOHOLS TO PICOLINATES – A NEW OPTION FOR HPLC ANALYSIS WITH CHIRAL STATIONARY PHASE†

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Abstract – Derivatization of secondary alcohols (R^1R^2CHOH) to benzoates has frequently employed to determine enantiomer ratios using HPLC with chiral stationary phase (CSP). However, a small difference in substituents (R^1, R^2) often results in insufficient separation. To find an alternative derivatization that detects such a small difference, picolinates (2-pyridyl-$CO_2CHR^1R^2$) possessing Me/Et, Me/vinyl, Me/acetylenic, Et/*n*-Pr, and *n*-Pr/allyl substituents were prepared and separation efficiency was compared with that of benzoates ($PhCO_2CHR^1R^2$). Eight commercially available CSPs containing carbamates or benzoates of cellulose and amylose were examined to find that retention factors (k'_1 and k'_2) and resolution (R_s) of picolinates were greater than those of the corresponding benzoates and that good to excellent R_s values ($\geq$1.25) were recorded over a wide range of CSPs.

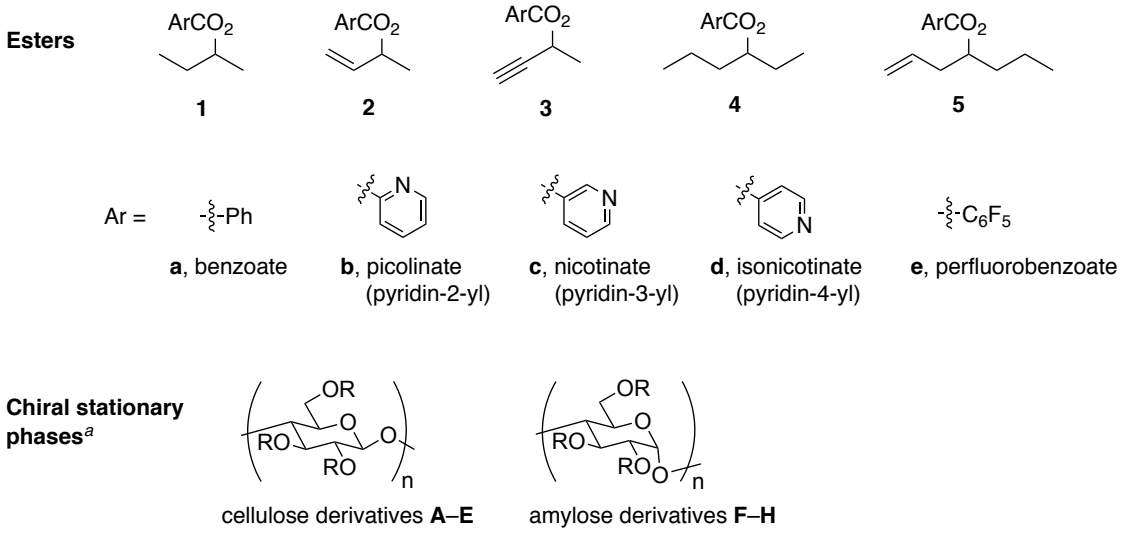
INTRODUCTION

Currently, many HPLC with chiral stationary phase (CSP) are commercially available for analysis of enantiomeric ratios of enantioenriched compounds.[1–4] For secondary alcohols, derivatization to benzoate is a frequently employed method. However, finding an appropriate chiral column for a given benzoate is a tedious and time-consuming task when the difference between two substituents of a secondary alcohol is small. To find another derivative by which such small differences are detectable, substituted benzoates were subjected to HPLC analysis using a cellulose tribenzoate beads as a CSP,[5] and 4-methyl- and 4-methoxybenzoates provided the best selectivity in terms of separation factor (α) and retention factor ($k'_{1,2}$).[6] However, resolution (R_s) was not determined and the efficiency of these substituted benzoates on

† This paper is dedicated to Professor Kiyoshi Tomioka on the occasion of his 70th birthday.

frequently used chiral columns was not examined. Herein, we report derivatization of a series of alcohols to picolinates, their HPLC analysis using eight CSPs, and their performance by resolution (R_s).

We have studied allylic substitution at secondary carbon with organometallic reagents to find high reactivity and anti S_N2' selectivity with allylic picolinates (2-pyridylcarboxylates).[7–9] Chelation of the carbonyl oxygen and the pyridyl nitrogen in the picolinoxy moiety to $MgBr_2$, produced in situ from ArMgBr and $CuBr \cdot Me_2S$, is a plausible mechanism for the high reactivity and selectivity. Enantiomeric ratios of these picolinates have frequently been determined as such by HPLC with CSP. Observed long retention times were supposed by chelation of the picolinoxy group to the acidic carbamoyl hydrogen in CSPs, π-π stacking between the pyridyl group and the aromatic part of CSPs, dipole moment-affinity, and/or interaction of the allylic olefin with CSPs. Based on these suppositions, it was envisaged that secondary alcohols possessing slightly different alkyl substituents would be more clearly resolved by derivatization to picolinates rather than to the corresponding benzoates (Figure 1). On the other hands, several hydroxy steroids have been changed to picolinates for LC-ESI-MS spectroscopy.[10–12] However, these studies have aimed to develop high sensitivity method, which was different from our study.



[a] **A**, cellulose tris(3,5-dimethylphenylcarbamate) (coated on silica gel, Chiralcel OD-H); **B**, as above (immobilized to silica gel, Chiralpak IB); **C**, cellulose tris(3,5-dichlorophenylcarbamate) (immobilized to silica gel, Chiralpak IC); **D**, cellulose tribenzoate (coated on silica gel, Chiralcel OB-H); **E**, cellulose tris(4-methylbenzoate) (coated on silica gel, Chiralcel OJ-H); **F**, amylose tris(3,5-dimethylphenylcarbamate) (coated on silica gel, Chiralpak AD-H); **G**, as above (immobilized to silica gel, Chiralpak IA); **H**, amylose tris[(*S*)-α-methylbenzylcarbamate] (coated on silica gel, Chiralpak AS-H).

Figure 1. Esters and chiral stationary phases in the present study

RESULTS AND DISCUSSION

Method. Esters **1a**–**e**, **2a**,**b**, **3a**,**b**, **4a**,**b**, and **5a**,**b** shown in Figure 1 were prepared from corresponding alcohols by standard esterification (PhCOCl and pyridine, or acid, 2-chloro-1-methylpyridinium iodide,

DMAP, and Et_3N) in good yields. Picolinates thus prepared were quite stable, and allowed easy handling and purification by chromatography for HPLC analysis. No byproduct(s) containing a picolinic acid moiety was co-produced and UV detection was operated without any interfere. HPLC analysis of these esters was carried out using cellulose- and amylose-based CSPs **A**–**E** and **F**–**H**, respectively, with hexane/*i*-PrOH (99:1) as an eluent at a flow rate of 1 mL/min at 35 °C, unless otherwise noted, to obtain the following data: net retention time (t_1–t_0) to assess polarity; retention factors (k'_1 and k'_2 for the first and second peaks) to provide the strength of interaction between the CSP and the ester; separation factor (α), which indicates the enantiomer resolving power of the CSPs; and resolution (R_s) to evaluate the efficiency of peak separation. Since k'_1 values for the first peaks of benzoates **1a**–**5a** were, in most cases, below the preferable range of 1–10,[13] the benzoates were also eluted with hexane to attain larger k'_1. R_s was calculated according to eq. (1), in which peak widths ($w_{1,2}$) were computed. Retention time of a baseline disturbance was used as the hold-up time (t_0) since the retention times of some entries were almost the same as those of 1,3,5-(t-Bu)$_3$C$_6$H$_3$. In theory, mutual overlaps of peaks at 1.5, 1.25, and 1.0 of R_s are 0.15%, 0.5%, and 2%, respectively, and thus peak separation in this study was rated by R_s as excellent ($R_s \geq 1.5$), good ($1.5 > R_s \geq 1.25$), partial ($1.25 > R_s \geq 1.0$), or overlap ($R_s < 1.0$).[13] In contrast to R_s, separation factor (α) is not directly indicative of resolution, and indeed, a somewhat low correlation between α and R_s was calculated as shown in Figures S1–S5 in the Supporting Information.

$$R_s = 2(t_2 - t_1)/(w_1 + w_2) \qquad (1)$$

$$R_s = 1.18(t_2 - t_1)/(w_{1,h/2} + w_{2,h/2}) \qquad (2)$$

Preliminary Analysis. Chromatograms and retention times (t_1, t_2) of picolinate **2b** using CSP **A** and those of benzoates **1a** and **4a** using CSPs **D** and **F** were found in literatures.[14,15] We calculated R_s values from the chromatograms according to eq. (2) using the width at half-height ($w_{h/2}$). For comparison, HPLC analysis of these esters with the same CSPs was repeated using our HPLC system under the published conditions to obtain following retention times (t_1 and t_2) and resolution (R_s). These data were more or less the same as the published values, and the performance of our CSPs **A**, **D**, and **F** was considered to be reproduced. On the other hand, the slight differences were primarily due to aged deterioration, suggesting that other columns were similarly aged. Consequently, the present results would be valuable for choosing an appropriate CSP from a CSP stock.

- for **2b** using **A** (hexane/*i*-PrOH (95:5), 1.2 mL/min): $t_{1,2}$ = 7.43, 8.13; R_s = 2.36; lit.[14] $t_{1,2}$ = 6.79, 7.28; R_s = 1.96.
- for **1a** using **D** (hexane, 1 mL/min): $t_{1,2}$ = 5.95, 6.34; R_s = 1.66; lit.[15] $t_{1,2}$ = 7.58, 8.18; R_s = 1.89;

- for **4a** using **F** (hexane/*i*-PrOH (99:1), 0.5 mL/min): $t_{1,2}$ = 10.13, 10.50; R_s = 0.93; lit.[15] $t_{1,2}$ = 9.24, 9.54; R_s = 1.14.

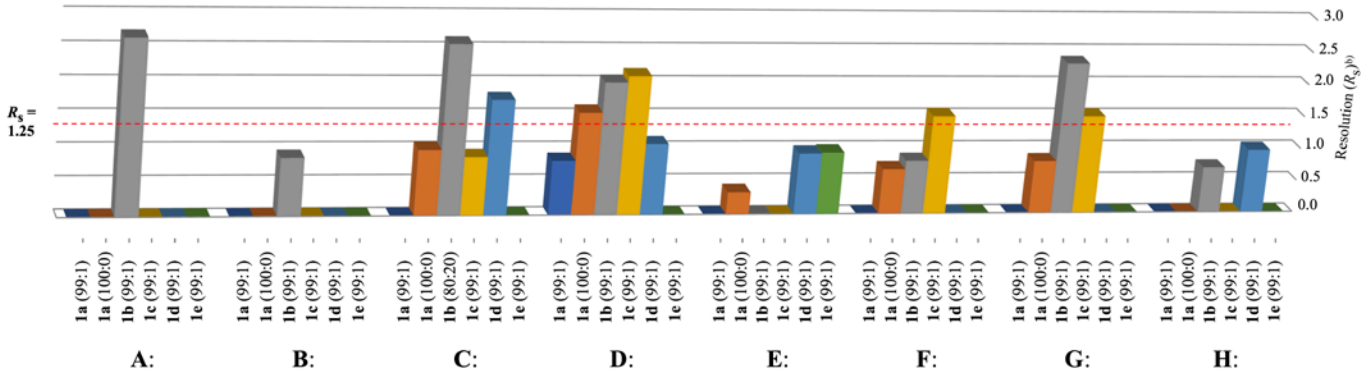
Analysis of esters 1a–1e derived from butan-2-ol. First, esters of butan-2-ol were chosen for HPLC study because the Me and Et substituents are differentiated only by size. As summarized in Table 1, enantiomers of picolinate **1b** were eluted with reasonable values of k'_1 (≥1.79) for the first peak in all cases (entries 17–24) and excellent R_s (≥ 2.01) was obtained with CSPs **A**, **C**, **D**, **G** (entries 17, 19, 20, 23). In contrast, k'_1 and R_s of benzoate **1a** were below 1 and 1.25, respectively. Elution with hexane yielded k'_1 values larger than 1 for CSPs **A**–**D**, **F** and **G**. However, only **D** showed excellent R_s of 1.56 (entry 8). Relationship between R_s of **1a**,**b** and CSPs (**A**–**H**) is graphically shown in Figure 2 as well.

Table 1. HPLC analysis of esters **1a** and **1b** using CSPs

entry	ester	CSPs	H:IPA[a]	$t_1 - t_0$	k'_1	k'_2	α[b]	R_s[c]	evaluation of R_s[d]
1	**1a**	**A**	99:1	1.13	0.35	0.35	1.00	0.00	overlap
2	**1a**	**A**	100:0	6.40	2.12	2.12	1.00	0.00	overlap
3	**1a**	**B**	99:1	0.73	0.23	0.23	1.00	0.00	overlap
4	**1a**	**B**	100:0	4.83	1.51	1.51	1.00	0.00	overlap
5	**1a**	**C**	99:1	1.74	0.55	0.55	1.00	0.00	overlap
6	**1a**	**C**	100:0	30.53	8.90	9.58	1.08	0.99	overlap
7	**1a**	**D**	99:1	1.11	0.37	0.44	1.16	0.83	overlap
8[e]	**1a**	**D**	100:0	2.90	0.95	1.08	1.13	1.56[f]	excellent
9	**1a**	**E**	99:1	1.20	0.38	0.38	1.00	0.00	overlap
10	**1a**	**E**	100:0	2.83	0.89	0.93	1.05	0.34	overlap
11	**1a**	**F**	99:1	1.63	0.52	0.52	1.00	0.00	overlap
12	**1a**	**F**	100:0	4.32	1.32	1.40	1.06	0.69	overlap
13	**1a**	**G**	99:1	1.22	0.40	0.40	1.00	0.00	overlap
14	**1a**	**G**	100:0	4.29	1.39	1.46	1.05	0.81	overlap
15	**1a**	**H**	99:1	0.48	0.16	0.16	1.00	0.00	overlap
16	**1a**	**H**	100:0	1.51	0.49	0.49	1.00	0.00	overlap
17	**1b**	**A**	99:1	14.07	4.66	5.21	1.12	2.66	excellent
18	**1b**	**B**	99:1	14.04	4.58	4.79	1.05	0.88	overlap
19	**1b**	**C**	80:20[g]	8.98	2.89	3.24	1.12	2.58	excellent
20	**1b**	**D**	99:1	18.53	6.03	6.63	1.10	2.01	excellent
21	**1b**	**E**	99:1	7.68	2.47	2.47	1.00	0.00	overlap
22	**1b**	**F**	99:1	14.74	4.85	5.03	1.04	0.82	overlap
23	**1b**	**G**	99:1	12.21	3.95	4.55	1.15	2.32	excellent
24	**1b**	**H**	99:1	5.44	1.79	1.88	1.05	0.70	overlap

[a] Ratio of hexane/*i*-PrOH. [b] Correlation between α and R_s is shown in the Supporting Information. [c] Calculated by eq. (1). [d] Excellent, R_s ≥1.5; good, 1.5> R_s ≥1.25; partial 1.25> R_s ≥1.0; or overlap (R_s <1.0). [e] t_1, t_2 = 5.95, 6.34. [f] R_s by eq. (2) = 1.66. [g] H:IPA (99:1) gave a longer elution time.

Next, HPLC analysis of nicotinate **1c** and isonicotinate **1d** disclosed values of k'_1 that were large enough in most cases (>1.0) (Table 2). However, the values were smaller than those of picolinate **1b**. Sufficient peak separation (R_s ≥1.25) was attained for **1c** using CSPs **D**, **F**, **G** (entries 4, 6, 7), while **1d** was separated only by **C** (entry 11). Relationship between R_s and CSPs is also shown in Figure 2.



[a] Ratios of hexane:*i*-PrOH are given in parentheses. [b] R_s of ≥1.25 indicate good to excellent separation.

Figure 2. Resolution (R_s) of esters **1a**–**1e**[a]

Since the pyridyl moiety in **1b**–**d** was more polar than the phenyl group in **1a**, perfluorobenzoate **1e** was also subjected to HPLC analysis (Table 2, entries 17–24). However, partial (<1.0) or almost marginal resolution was observed, and t_1–t_0 and $k'_{1,2}$ were lower than those of benzoate **1a**, indicating little interaction between **1e** and the CSPs.

Table 2. HPLC analysis of esters **1c**, **1c**, and **1e** using CSPs

entry	ester	CSPs	H:IPA[a]	$t_1 - t_0$	k'_1	k'_2	α[b]	R_s[c]	evaluation of R_s[d]
1	**1c**	**A**	99:1	7.06	2.29	2.29	1.00	0.00	overlap
2	**1c**	**B**	99:1	4.14	1.34	1.34	1.00	0.00	overlap
3	**1c**	**C**	99:1	32.54	10.00	10.40	1.04	0.89	overlap
4	**1c**	**D**	99:1	2.87	0.94	1.09	1.16	2.11	excellent
5	**1c**	**E**	99:1	2.70	0.85	0.85	1.00	0.00	overlap
6	**1c**	**F**	99:1	7.25	2.32	2.51	1.08	1.50	excellent
7	**1c**	**G**	99:1	6.71	2.24	2.42	1.08	1.50	excellent
8	**1c**	**H**	99:1	2.39	0.79	0.79	1.00	0.00	overlap
9	**1d**	**A**	99:1	4.53	1.51	1.51	1.00	0.00	overlap
10	**1d**	**B**	99:1	4.07	1.23	1.23	1.00	0.00	overlap
11	**1d**	**C**	99:1	22.43	7.09	7.55	1.06	1.75	excellent
12	**1d**	**D**	99:1	3.00	0.98	1.06	1.08	1.08	partial
13	**1d**	**E**	99:1	3.84	1.17	1.25	1.07	0.93	overlap
14	**1d**	**F**	99:1	5.83	1.85	1.85	1.00	0.00	overlap
15	**1d**	**G**	99:1	4.77	1.53	1.53	1.00	0.00	overlap
16	**1d**	**H**	99:1	2.73	0.88	1.00	1.13	0.98	overlap
17	**1e**	**A**	99:1[e]	0.95	0.30	0.30	1.00	0.00	overlap[e]
18	**1e**	**B**	99:1[e]	0.60	0.19	0.19	1.00	0.00	overlap[e]
19	**1e**	**C**	99:1[e]	0.67	0.20	0.20	1.00	0.00	overlap[e]
20	**1e**	**D**	99:1[e]	0.50	0.16	0.16	1.00	0.00	overlap[e]
21	**1e**	**E**	99:1[e]	0.58	0.17	0.22	1.30	0.95	overlap[e]
22	**1e**	**F**	99:1[e]	0.88	0.28	0.28	1.00	0.00	overlap[e]
23	**1e**	**G**	99:1[e]	0.81	0.26	0.26	1.00	0.00	overlap[e]
24	**1e**	**H**	99:1[e]	0.27	0.09	0.09	1.00	0.00	overlap[e]

[a–d] See footnotes *a*–*d* of Table 1. [e] Elution with hexane gave R_s of ≤1.08 and longer t_1 and t_2, which were, in turn, shorter than those of **1a**.

In summary of the above study (Tables 1 and 2, Figure 2), potential for peak separation was decisively

increased by introduction of the pyridyl ring, but the performance was dependent on the position of nitrogen. Thus, R_s was in the order of **1b** ≥ **1c**> **1d** ≥ **1a** > **1e**. Hydrogen bonding is most likely for binding the picolinoxy group to the acidic hydrogen on the carbamoyl nitrogen to produce a sufficient structural interaction between a picolinate and a carbamate CSP. However, other CSPs, which gave low R_s, indicated that affinity was influenced by another factor(s). Alternatively, CSP **D**, possessing the benzoyl group, efficiently separated **1b** (Table 1, entry 20) and **1c** (Table 2, entry 4). These results imply that π-π stacking and/or dipole interaction were also responsible for the tight binding of the picolinate to the CSP.[16–19] On the basis of R_s and k', it was concluded that **1b** is the better ester than **1c**,**d** and **1a**,**e** for CSPs **A**–**H**.

Analysis of other picolinates and benzoates. HPLC analysis of picolinate **2b** and benzoate **2a** derived from but-3-en-2-ol are presented in Table 3. Retention factors ($k'_{1,2}$) and resolution (R_s) of **2b** were larger than those of picolinate **1b** and good to excellent R_s was observed using **A**, **B**, **C**, **F**, and **G** (entries 17–19, 22, 23). As for benzoate **2a**, excellent R_s was obtained using CSPs **D** and **F** (entries 7, 11). As expected, $k'_{1,2}$ of **2a** were improved by eluting with hexane and good to excellent R_s values were recorded with CSPs **A**, **C**, **D**, **F**, **G** (entries 2, 6, 8, 12, 14).

Table 3. HPLC analysis of esters **2a** and **2b** using CSPs

entry	ester	CSPs	H:IPA[a]	$t_1 - t_0$	k'_1	k'_2	α[b]	R_s[c]	evaluation of R_s[d]
1	**2a**	**A**	99:1	1.48	0.45	0.45	1.00	0.00	overlap
2	**2a**	**A**	100:0	7.61	2.52	2.77	1.10	1.91	excellent
3	**2a**	**B**	99:1	0.86	0.27	0.27	1.00	0.00	overlap
4	**2a**	**B**	100:0	5.89	1.84	1.93	1.04	0.67	overlap
5	**2a**	**C**	99:1	1.96	0.67	0.74	1.10	1.21	partial
6	**2a**	**C**	100:0	30.11	9.03	9.81	1.09	1.33	good
7	**2a**	**D**	99:1	2.03	0.67	0.77	1.16	1.68	excellent
8	**2a**	**D**	100:0	5.18	1.69	2.18	1.29	4.03	excellent
9	**2a**	**E**	99:1	2.06	0.63	0.63	1.00	0.00	overlap
10	**2a**	**E**	100:0	4.45	1.41	1.48	1.05	0.62	overlap
11	**2a**	**F**	99:1	1.83	0.58	0.66	1.15	1.51	excellent
12	**2a**	**F**	100:0	5.02	1.55	1.82	1.18	2.58	excellent
13	**2a**	**G**	99:1	1.28	0.38	0.43	1.13	1.12	partial
14	**2a**	**G**	100:0	4.90	1.57	1.85	1.17	2.89	excellent
15	**2a**	**H**	99:1	0.62	0.20	0.20	1.00	0.00	overlap
16	**2a**	**H**	100:0	1.97	0.64	0.72	1.13	0.84	overlap
17[e]	**2b**	**A**	99:1	18.57	6.11	7.34	1.20	4.41[e]	excellent
18	**2b**	**B**	99:1	17.82	5.62	6.08	1.08	1.49	good
19	**2b**	**C**	80:20[f]	10.33	3.23	5.09	1.58	10.68	excellent
20	**2b**	**D**	99:1	29.47	9.53	9.66	1.01	0.05	overlap
21	**2b**	**E**	99:1	12.25	3.74	3.74	1.00	0.00	overlap
22	**2b**	**F**	99:1	19.24	6.18	6.57	1.06	1.56	excellent
23	**2b**	**G**	99:1	15.31	5.02	5.40	1.08	1.67	excellent
24	**2b**	**H**	99:1	7.58	2.18	2.25	1.03	0.24	overlap

[a–d] See footnotes *a*–*d* of Table 1. [e] Elution with H:IPA (95:5) at 1.2 mL/min gave: t_1, t_2 = 7.43, 8.13; R_s = 2.27 by eq. (1) and 2.36 by eq. (2). [f] H:IPA (99:1) gave a longer elution time.

Picolinate **3b** and benzoate **3a** possessing the alkynyl moiety were also subjected to HPLC analysis (Table 4). Most of the CSPs showed increased affinity to **3b** as assessed by $k'_{1,2}$ and R_s, and good to excellent R_s values were obtained for CSPs **A**–**E** (entries 17–21). Similarly, k' and R_s of benzoate **3a** were increased and CSPs **A**, **C**, **D**, **E**, **F**, and **G** gave good to excellent R_s even with hexane/*i*-PrOH (99:1) (entries 1, 5, 7, 9, 11, and 13). The use of hexane as the eluent added CSPs **B** and **H** to the excellent group of the CSPs (entries 4 and 16). These results clearly indicated that the alkynyl moiety was a more efficient chromophore than the alkenyl, suggesting an alkynyl-dependent mechanism for the tight binding to these CSPs.

Table 4. HPLC analysis of esters **3a** and **3b** using CSPs

entry	ester	CSPs	H:IPA[a]	$t_1 - t_0$	k'_1	k'_2	α[b]	R_s[c]	evaluation of R_s[d]
1	**3a**	**A**	99:1	2.42	0.82	0.93	1.15	1.82	excellent
2	**3a**	**A**	100:0	12.09	4.00	5.31	1.33	5.39	excellent
3	**3a**	**B**	99:1	1.52	0.43	0.49	1.13	0.75	overlap
4	**3a**	**B**	100:0	9.91	3.09	3.51	1.14	1.87	excellent
5	**3a**	**C**	99:1	2.92	0.91	1.07	1.18	2.55	excellent
6	**3a**	**C**	100:0	43.61	13.18	19.62	1.49	3.33	excellent
7	**3a**	**D**	99:1	5.78	1.84	2.01	1.09	1.51	excellent
8	**3a**	**D**	100:0	13.87	4.50	5.13	1.14	2.16	excellent
9	**3a**	**E**	99:1	5.56	1.68	1.92	1.14	2.41	excellent
10	**3a**	**E**	100:0	10.90	3.43	3.83	1.12	1.93	excellent
11	**3a**	**F**	99:1	2.93	0.95	1.16	1.22	2.87	excellent
12	**3a**	**F**	100:0	7.60	2.34	2.99	1.28	3.28	excellent
13	**3a**	**G**	99:1	2.50	0.73	0.81	1.11	1.35	good
14	**3a**	**G**	100:0	7.44	2.41	3.06	1.27	3.58	excellent
15	**3a**	**H**	99:1	1.34	0.42	0.49	1.17	1.04	partial
16	**3a**	**H**	100:0	3.85	1.26	1.59	1.27	1.87	excellent
17	**3b**	**A**	99:1	31.36	9.93	14.07	1.42	9.70	excellent
18	**3b**	**B**	99:1	28.06	8.99	10.95	1.22	5.04	excellent
19	**3b**	**C**	80:20[e]	11.83	3.79	10.68	2.82	24.52	excellent
20	**3b**	**D**	99:1	75.62	24.64	32.21	1.31	7.13	excellent
21	**3b**	**E**	99:1	33.93	10.82	12.71	1.18	3.81	excellent
22	**3b**	**F**	99:1	29.55	9.06	9.34	1.03	0.73	overlap
23	**3b**	**G**	99:1	24.21	7.66	7.86	1.03	0.45	overlap
24	**3b**	**H**	99:1	14.82	4.65	5.04	1.08	0.90	overlap

[a–d] See footnotes *a*–*d* of Table 1. [e] H:IPA (99:1) gave a longer elution time.

Picolinate **4b** and benzoate **4a** were then prepared next from hexan-3-ol, in which a combination of the Et and *n*-Pr substituents represents a group of smaller size difference than that of **1a**,**b**. Enantiomers of **4b** were separated with CSPs **A**, **B**, **C**, and **G** (Table 5, entries 17–19, 23). For benzoate **4a**, retention factors ($k'_{1,2}$) were lower than for **1a** possessing Me and Et substituents, and the R_s values were ≤089. When eluted with hexane, **D** and **G** could separate **4a** with excellent R_s (entries 8, 14).

Table 5. HPLC analysis of esters **4a** and **4b** using CSPs

entry	ester	CSPs	H:IPA[a]	$t_1 - t_0$	k'_1	k'_2	α[b]	R_s[c]	evaluation of R_s[d]
1	**4a**	**A**	99:1	0.76	0.26	0.26	1.00	0.00	overlap
2	**4a**	**A**	100:0	5.23	1.73	1.73	1.00	0.00	overlap
3	**4a**	**B**	99:1	0.52	0.17	0.17	1.00	0.00	overlap
4	**4a**	**B**	100:0	4.37	1.37	1.37	1.00	0.00	overlap
5	**4a**	**C**	99:1	1.33	0.40	0.45	1.11	0.71	overlap
6	**4a**	**C**	100:0	22.74	6.86	7.38	1.08	1.19	partial
7	**4a**	**D**	99:1	0.90	0.28	0.32	1.14	0.44	overlap
8	**4a**	**D**	100:0	2.53	0.82	0.99	1.21	1.71	excellent
9	**4a**	**E**	99:1	0.74	0.24	0.24	1.00	0.00	overlap
10	**4a**	**E**	100:0	1.69	0.53	0.53	1.00	0.00	overlap
11[e]	**4a**	**F**	99:1	1.04	0.34	0.39	1.15	0.89[e]	overlap
12	**4a**	**F**	100:0	3.77	1.17	1.29	1.10	1.08	partial
13	**4a**	**G**	99:1	0.91	0.30	0.33	1.09	0.34	overlap
14	**4a**	**G**	100:0	4.09	1.32	1.48	1.12	1.53	excellent
15	**4a**	**H**	99:1	0.31	0.10	0.10	1.00	0.00	overlap
16	**4a**	**H**	100:0	0.96	0.31	0.33	1.06	0.00	overlap
17	**4b**	**A**	99:1	8.64	2.92	3.47	1.19	3.58	excellent
18	**4b**	**B**	99:1	9.34	3.11	3.34	1.07	1.51	excellent
19	**4b**	**C**	80:20[f]	6.63	2.06	2.20	1.07	1.31	good
20	**4b**	**D**	99:1	15.00	4.83	4.83	1.00	0.00	overlap
21	**4b**	**E**	99:1	4.45	1.42	1.54	1.08	1.06	partial
22	**4b**	**F**	99:1	11.51	3.74	3.74	1.00	0.00	overlap
23	**4b**	**G**	99:1	10.35	3.45	3.72	1.08	1.71	excellent
24	**4b**	**H**	99:1	3.16	1.02	1.02	1.00	0.00	overlap

[a–d] See footnotes *a–d* of Table 1. [e] A different flow rate of 0.5 mL/min gave: t_1, t_2 = 10.13, 10.50; R_s = 0.92 by eq. (1) and 0.93 by eq. (2). [f] H:IPA (99:1) gave a longer elution time.

Finally, picolinate **5b** and benzoate **5a** possessing an olefin moiety on one substituent were subjected to HPLC analysis to obtain results as shown in Table 6. Retention factors ($k'_{1,2}$) of picolinate **5b** were decreased, and CSPs **A** and **D** showed excellent R_s (entries 17 and 20). The R_s variation patterns for CSPs/**5b** were different from those for CSPs/**1b**,**2b** (Tables 1 and 3, entries 17–24 for the both tables). Benzoate **5a** was separated by CSP **F** using hexane/*i*-PrOH (99:1) (entry 11) and by **A** using hexane (entry 2).

CONCLUSIONS

Picolinates and benzoates derived from several secondary alcohols were analyzed by HPLC with eight CSPs containing carbamates or benzoates of cellulose. Picolinates were readily prepared from the alcohols by mixing with picolinic acid (2-pyridyl-CO_2H), 2-chloro-1-methylpyridinium iodide, DMAP, and Et_3N in CH_2Cl_2 at room temperature for several hours. All the reagents were obtained easily. Retention factors ($k'_{1,2}$) and resolution (R_s) of the picolinates were larger than those of the benzoates: good to excellent R_s values (≥1.25) were obtained with several CSPs. Furthermore, the picolinates showed high affinity to CSPs of carbamate- and benzoate-types. Acetylenic moiety in a substituent showed high affinity to the CSPs. In conclusion, the results presented herein indicate that derivatization to picolinates

could be a new option for HPLC analysis of secondary alcohols using CSPs.

Table 6. HPLC analysis of esters **5a** and **5b** using CSPs

entry	ester	CSPs	H:IPA[a]	$t_1 - t_0$	k'_1	k'_2	α[b]	R_s[c]	evaluation of R_s[d]
1	**5a**	**A**	99:1	0.82	0.27	0.29	1.01	0.02	overlap
2	**5a**	**A**	100:0	5.34	1.77	2.15	1.22	3.59	excellent
3	**5a**	**B**	99:1	0.50	0.16	0.16	1.00	0.00	overlap
4	**5a**	**B**	100:0	4.69	1.47	1.60	1.09	1.23	partial
5	**5a**	**C**	99:1	1.24	0.38	0.38	1.00	0.00	overlap
6	**5a**	**C**	100:0	23.35	7.07	7.69	1.09	1.18	partial
7	**5a**	**D**	99:1	1.10	0.36	0.40	1.11	0.12	overlap
8	**5a**	**D**	100:0	3.71	1.20	1.20	1.00	0.00	overlap
9	**5a**	**E**	99:1	0.85	0.26	0.26	1.00	0.00	overlap
10	**5a**	**E**	100:0	1.87	0.59	0.62	1.06	0.25	overlap
11	**5a**	**F**	99:1	1.54	0.49	0.58	1.17	1.34	good
12	**5a**	**F**	100:0	4.41	1.38	1.42	1.03	0.10	overlap
13	**5a**	**G**	99:1	1.04	0.34	0.38	1.09	0.35	overlap
14	**5a**	**G**	100:0	4.75	1.53	1.60	1.05	0.46	overlap
15	**5a**	**H**	99:1	0.34	0.11	0.11	1.00	0.00	overlap
16	**5a**	**H**	100:0	1.17	0.38	0.42	1.11	0.38	overlap
17	**5b**	**A**	99:1	9.27	3.10	3.39	1.09	1.80	excellent
18	**5b**	**B**	99:1	8.75	2.79	2.90	1.04	0.80	overlap
19	**5b**	**C**	80:20[e]	5.88	1.83	1.91	1.04	0.62	overlap
20	**5b**	**D**	99:1	17.23	5.63	8.57	1.52	4.35	excellent
21	**5b**	**E**	99:1	5.07	1.57	1.70	1.08	1.13	partial
22	**5b**	**F**	99:1	14.37	4.61	4.85	1.05	1.19	partial
23	**5b**	**G**	99:1	12.36	4.08	4.08	1.00	0.00	overlap
24	**5b**	**H**	99:1	3.58	1.18	1.18	1.00	0.00	overlap

[a–d] See footnotes *a–d* of Table 1. [e] H:IPA (99:1) gave a longer elution time.

EXPERIMENTAL

HPLC analysis of esters using CSPs was performed using hexane/*i*-PrOH (99/1) or hexane as an eluent unless otherwise specified. Signals were processed using the LC solution (version 1.25 SP1). Resolution (R_s) was calculated by eq. (1) using computed baseline peak widths (w_1 and w_2). For comparison of R_s with those in the literatures,[14,15] R_s values calculated by eq. (2) were used because calculation of $w_{1,h/2}$ and $w_{2,h/2}$ (the width at half-height) from the chromatograms attached in the literatures were easier and more accurate by us than that of w_1 and w_2.

Benzoates **1a**–**5a** were synthesized from the corresponding alcohols with PhCOCl in pyridine. Picolinates **1b**–**5b** and other esters **1c**–**e** were prepared by esterification with the corresponding acids using 2-chloro-1-methylpyridinium iodide, *N*,*N*-dimethyl-4-aminopyridine (DMAP), and Et_3N in CH_2Cl_2. Procedures, characterization of esters, and copies of NMR spectra were given in the Supporting Information.

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