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Note

Enantiomeric separation of racemic pterocarpans by high-performance liquid chromatography on (+)-poly(triphenylmethyl methacrylate)-coated silica gel

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In recent years, the use of high-performance liquid chromatography (HPLC) for the separation of enantiomers increased rapidly as a number of chiral stationary phases became commercially available[1–4] and some of them have been proposed for the separation of enantiomeric flavonoids. Okamoto *et al.*[5] successfully separated a racemic mixture of the unsubstituted flavanone on cellulose triphenylcarbamate supported on silica gel (Chiralcel OC; Daicel, Japan). Krause and Galensa[6] used microcrystalline cellulose triacetate as a chiral stationary phase for the separation of racemic polyhydroxy flavanones. The optical resolution of chalcone epoxides and flavanols has also been achieved by using a helical poly(triphenylmethyl methacrylate)-coated silica gel [Chiralpack OT (+); Daicel][7]. This chiral stationary phase has also shown an extraordinary selectivity for many enantiomers of rotenoids possessing a rigid non-planar structure[8].

As the naturally occurring pterocarpans, a group of compounds with phytoalexin properties, also possess a rigid non-planar structure due to *cis* B/C ring fusion, it was considered of interest to study the separation of corresponding enantiomeric mixtures on helical (+)-poly(triphenylmethyl methacrylate)-modified silica gel. In this paper we report the enantiomeric separation of synthetic and naturally occurring racemic pterocarpans (Fig. 1) and the influence of their substitution patterns on chromatographic resolution.

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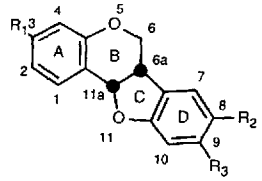

	R_1	R_2	R_3
1	H	H	H
2	OH	H	H
3	OH	H	OEt
4	OMe	H	OH
5	OH	$-OCH_2O-$	
6	OH	H	OMe
7	OMe	H	OMe
8	OnPr	H	OMe
9	OCH_2CO_2Et	H	OMe
10	$O(CH_2)_{10}CO_2Et$	H	OMe
11	OCH_2CH_2OH	H	OMe
12	$O(CH_2)_{10}CH_2OH$	H	OMe
13	OAc	H	OMe
14	$OCOCH_2CH_3$	H	OMe
15	$OCO(CH_2)_9CH_3$	H	OMe
16	OCH_2OMe	H	OMe
17	OCH_2OEt	H	OMe
18	THP	H	OMe

Fig. 1. Pterocarpans studied. Et = C_2H_5; Me = CH_3.

EXPERIMENTAL

Materials

The racemates of pterocarpan (**1**), 3-hydroxypterocarpan (**2**), 3-hydroxy-9-ethoxypterocarpan (**3**), 3-methoxy-9-hydroxypterocarpan (**4**), maackiain (**5**) and medicarpin (**6**) were synthesized by the thallium(III) nitrate method[9] starting from 2′-hydroxychalcones. Homopterocarpin (**7**) and other medicarpin derivatives (**8–18**) were prepared from **6**[10].

HPLC

The HPLC separation was carried out with a Hewlett-Packard HP 1090A instrument using an HP 1040A photodiode-array detection system and an HP 3392A integrator. Chiralpak OT (+), 250 mm × 4.6 mm (Daicel), was used as the stationary phase. The mobile phase was methanol (HPLC grade) at a regular flow-rate of 0.5 ml/min.

Photometric detection was performed at 280 nm. The injection volume was 10 μl (*ca.* 10 μg). Polarimetric detection was performed with an ACS ChiraMonitor

(Applied Chromatography Systems, Cheshire, U.K.) with a 20-μl flow cell of path length 20 mm using a 2-mW collimated near-IR laser diode (830 nm) as light source[11]. The injection volume was 50 μl (*ca.* 50 μg).

RESULTS AND DISCUSSION

Chromatographic data for the enantiomeric pterocarpan derivatives **1–18** on poly(triphenylmethyl methacrylate) using methanol as the mobile phase at 0.5 ml/min are given in Table I and Fig. 2. In this separation system most of the racemates were baseline resolved. In every instance the dextrorotatory enantiomers of 6a*S*, 11a*S* configuration[12,13] were eluted first. It is interesting that most of the known natural pterocarpans have large negative $[\alpha]_D$ values owing to the 6a*R*, 11a*R* absolute configuration, but homopterocarpin (**7**)[14], medicarpin (**6**)[14,15] and maackiain (**5**)[16,17] exist in nature in both antipodal forms and the last two also as racemates[14,17].

A very high resolution (R_s = 4.00) and separation factor (α = 2.43) were observed for the unsubstituted pterocarpan (**1**). This clearly showed that a strong π–π interaction between the aromatic parts of the substrate and the pendant trityl groups of the polymeric chain is the most important factor in the chiral recognition mechanism.

Owing to a possible hydrogen-bonding mechanism, this non-polar interaction can strongly be disturbed by the hydroxy substituent of the A-ring (**2**, R_s = 1.00) or by the primary alcoholic function of the side-chain connected at C-3 of the pterocarpan skeleton (**11**, **12**, R_s = 1.52 and 1.85, respectively). In the presence of an alkoxy group

TABLE I

CHROMATOGRAPHIC DATA FOR RACEMIC PTEROCARPANS

t_R = Retention time (min); R_s = resolution factor = 2 × (distance between the peaks of the enantiomers)/sum of band widths of the two peaks; k' = capacity factor = (retained volume of enantiomer − void volume of column)/void volume of column; α = separation factor = $k'(-)/k'(+)$.

Compound	$t_R(-)$	$k'(-)$	$t_R(+)$	$k'(+)$	α	R_s
1	23.36	5.31	11.77	2.18	2.44	4.00
2	11.14	2.01	9.21	1.49	1.35	1.00
3	13.85	2.74	9.47	1.56	1.76	1.91
4	12.50	2.38	9.25	1.50	1.59	2.00
5	11.31	2.06	9.32	1.52	1.35	2.20
6	12.17	2.29	8.97	1.42	1.61	2.10
7	19.19	4.19	13.54	2.66	1.57	2.40
8	22.92	5.19	14.66	2.96	1.75	3.03
9	22.48	5.08	14.08	2.81	1.81	2.80
10	42.35	10.45	24.55	5.64	1.85	2.04
11	12.92	2.49	9.84	1.66	1.50	1.52
12	20.95	4.66	13.70	2.70	1.73	1.85
13	13.78	2.72	11.41	2.08	1.31	1.00
14	15.32	3.14	11.16	2.02	1.56	1.75
15	23.40	5.32	14.39	2.89	1.84	1.83
16	20.41	4.52	12.98	2.51	1.80	2.92
17	19.67	4.32	13.04	2.52	1.71	2.12
18[a]	15.74/18.73	3.18/4.06	9.31/10.77	1.52/1.91	2.10/2.13	2.20/1.38

[a] Retention times with a flow-rate of 1.0 ml/min.

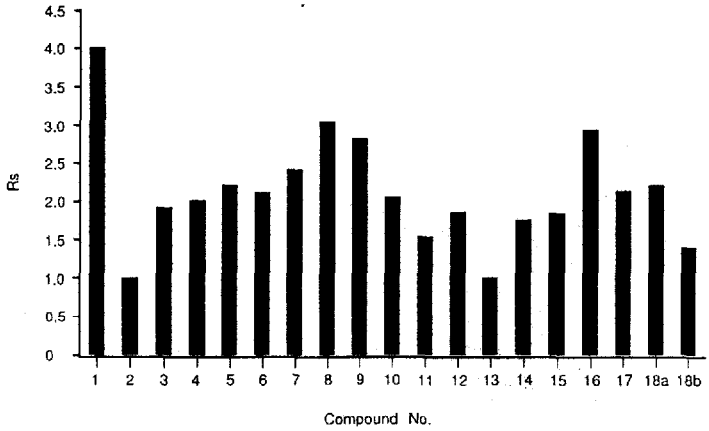

Fig. 2. Chromatographic resolution (R_s) of racemic pterocarpan derivatives on (+)-poly(triphenylmethyl methacrylate)-coated silica gel. Mobile phase: methanol at a flow-rate of 0.5 ml/min.

on the A- or D-ring, this effect decreases considerably. Therefore, an adequate resolution of the eantiomers was achieved for **3–6**. For maackiain (**5**) a resolution factor of 1.8 and very small capacity factors (0.166 and 0.3) have been reported by Zief[18].

On reducing the polar character of medicarpin (**6**) by etherification of the hydroxy group, the enantiomeric separation (R_s for **6** < **7** < **8** ≈ **16**) improved

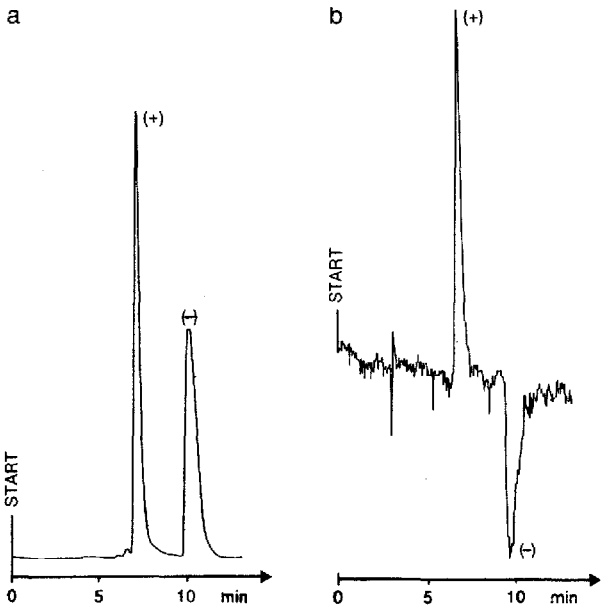

Fig. 3. HPLC separation of 46 μg 3-O-*n*-propylmedicarpin (**8**) on (+)-poly(triphenylmethyl methacrylate)-coated silica gel. Mobile phase: methanol at a flow-rate of 1.0 ml/min. Dual detection: (a) photometric detection at 280 nm (attenuation 256 × 0.01); (b) ACS ChiraMonitor (attenuation 4).

significantly (Fig. 3). The optimum length of the alkyl or alkoxy substituent seems to be *ca.* 2.5 Å, shown by the fact that elongation of the side-chain of **16** with one CH_2 group has resulted in a decreasing resolution factor (**16**, **17**, R_s = 2.92 and 2.12, respectively). The same effect was observed with ethoxycarbonylmethyl derivatives (**9**, **10**). In agreement with the findings of Takahashi *et al.*[7], replacement of the hydroxy or methoxy group on the aromatic ring by an acetoxy group also lowered the R_s values (**13**, R_s = 1.00). This unfavourable effect could be partly compensated for by the alkyl moiety of the ester group (R_s of **13** < **14** < **15**).

CONCLUSION

Poly(triphenylmethyl methacrylate) is a useful chiral stationary phase for resolving racemic hydroxypterocarpan derivatives. The efficiency of the separation is increased by derivatization of the hydroxy group with methoxymethyl or *n*-propyl functions. This technique can be applied to the optical resolution of synthetic or natural racemates of pterocarpans to obtain the corresponding optical isomers for studies of structure–activity relationships. Further investigations are in progress to study the chiral recognition mechanism with respect to the substitution pattern of pterocarpans.

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