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Enantiomeric separation of racemic isoflavanones and related compounds on (+)-poly(triphenylmethyl methacrylate)-coated silica gel

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ABSTRACT

(+)-Poly(triphenylmethyl methacrylate)-coated silica gel [Chiralpack OT(+)] was used for the separation of enantiomers of racemic isoflavanone, homoisoflavanone, isoflavan and 2-benzyltetralone derivatives. All the enantiomers of the above-mentioned compounds were separable. The efficiency of the separation depended on both the skeleton and substitution pattern of the racemic compounds. A relation between the number and polarity of substituents in the aromatic rings and the resolution and separation factors was observed.

INTRODUCTION

Isoflavanones, being the biosynthetic precursors of pterocarpan phytoalexins, are synthesized mostly by *Leguminosae* species in response to stress-induced microbial infection [1–5]. The stereochemistry of the isoflavanones involved in pterocarpan biosynthesis has been deduced from the configuration of the pterocarpans [6–8]. Thus, in the case of (6a*R*,11a*R*)-maackiain, the intermediate is (3*R*)-(+)-sophorol (Fig. 1).

3*R*-(+)-Sophorol [9], 3*S*-(−)-7,4′-dihydroxyisoflavanone [10], (−)-sophoraisoflavanone A [11], (−)-isosophoranone [12] and (−)-erosenone [13] are the only isoflavanones hitherto isolated from natural sources in optically active form. That the number of optically active isoflavanones is small is probably because they are rapidly racemized by keto–enol tautomerism in the course of their isolation.

Although isoflavanones have a less rigid conformational structure than the corresponding pterocarpans, a study of their Dreiding models [14] suggested that in one of their conformers, when the

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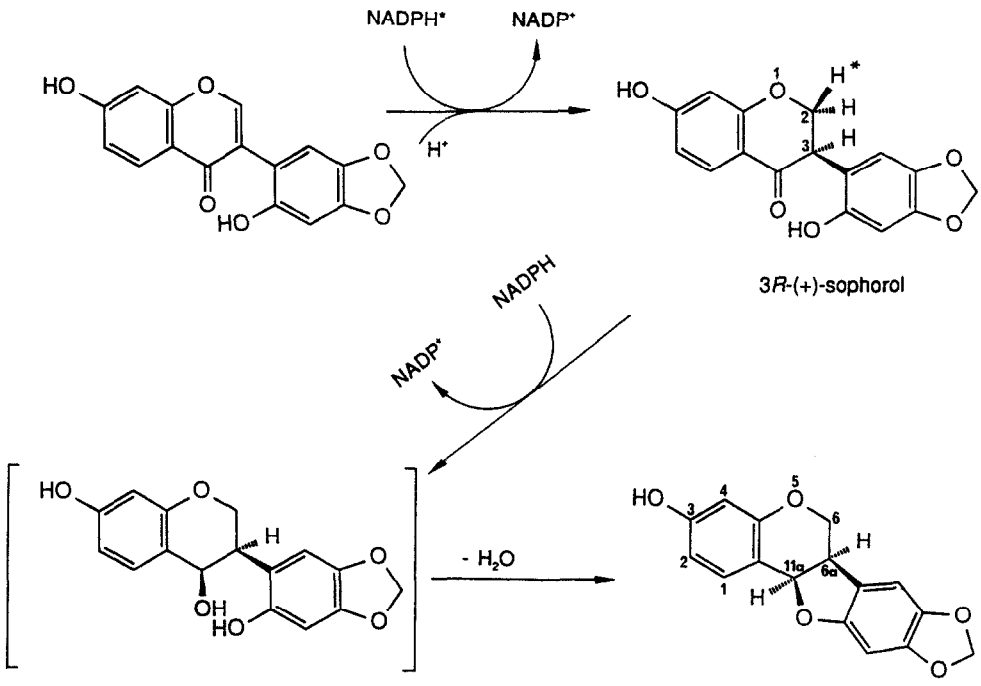


Fig. 1. Biosynthesis of (+)-maackiain.

aromatic ring at C-3 is in the *equatorial* position, the presumably dihedral angle is nearby, as it is in the homochiral pterocarpans.

Therefore, as a continuation of our work on the enantiomeric separation of pterocarpans [15], an efficient optical resolution of isovlavanones and related compounds would also be expected using (+)-poly(triphenylmethyl methacrylate) (PTrMA)-coated silica gel [Chiralpack OT(+)]. In this paper we report the enantiomeric separation of synthetic racemic isoflavanones and related compounds (Fig. 2) and the influence of their substitution patterns on the efficiency of the chromatographic resolution.

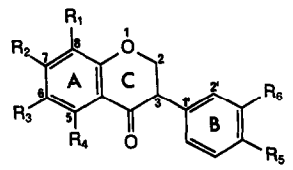
EXPERIMENTAL

Materials

The racemates of the isoflavanones **1**, **3–6**, **8–11** and **13** were synthesized from the appropriately substituted isoflavones by reduction with diisobutylaluminium hydride [16]. The same procedure was followed for synthesizing the homoisoflavanone **15** and 2-(4-methoxybenzyl)indanone (**16**) from the corresponding benzylidene derivatives. The isoflavanone derivatives **2**, **7** and **12** were prepared from **5**, **9** and **13** by acidic deprotection. Preparative separation of the enantiomers **1** [3*R*-(+)-**1**, 3*S*-(−)-**1**] and **14** [3*R*-(+)-**14**, 3*S*-(−)-**14**] was achieved via the isoflavanone dimethylethylene thioketals (**17a** and **17b**) [17] using the method of Corey and Mitra [18].

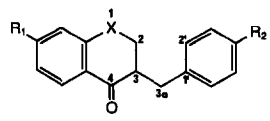
High-performance liquid chromatography (HPLC)

HPLC separation was carried out with a Hewlett-Packard HP 1090M liquid chromatograph with an HP 1040A photodiode-array detection system and an HP 3329A integrator. Chiralpack OT(+), 250 mm × 4.6 mm I.D. (Daicel), was used as the stationary phase. The mobile phase was methanol (HPLC grade) at a regular flow-rate of 0.5 ml/min (20°C). The injection volume was 10 μl (*ca.* 10 μg). The peaks of the enantiomeric separation of **1** and **14** were verified by co-chromatography of the (−)- and (+)-enantiomers.

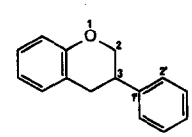


	R^1	R^2	R^3	R^4	R^5	R^6
1	H	H	H	H	H	H
2	H	OH	H	H	H	H
3	H	OMe	H	H	H	H
4	H	OiPr	H	H	H	H
5	H	OMEM	H	H	H	H
6	H	OMe	H	H	OMe	H
7	H	OH	H	OH	H	H
8	H	OMe	H	OMe	H	H
9	H	OBz	H	OBz	H	H
10	Me	OMe	H	OMe	H	H
11	H	OMe	OMe	OMe	H	H
12	H	OH	H	H	$-OCH_2O-$	
13	H	OMEM	H	H	$-OCH_2O-$	

Bz=CH_2Ph MEM=CH_2OMe



	X	R^1	R^2
15	O	OMe	OMe
16	CH_2	H	OMe



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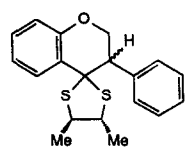
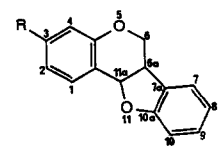


	3H
17a	α
17b	β



	R
18	H
19	OH

Fig. 2. Isoflavanones and related compounds studied.

RESULTS AND DISCUSSION

Chromatographic data for the enantiomers of isoflavanones **1–13** and related compounds (**14–16**) separated on PTrMA at room temperature using methanol as the mobile phase at 0.5 ml/min are given in Table I (formulae in Fig. 2).

To allow comparison of these data with those of

TABLE I

CHROMATOGRAPHIC DATA FOR RACEMIC ISOFLAVANONES AND RELATED COMPOUNDS

t_R = retention time (min); R_s = resolution factor = 2 × (distance between the peaks of the enantiomers/sum of band widths of two peaks); k' = capacity factor = (retained volume of enantiomer/void volume of column)/void volume of column; α = separation factor = k'_2/k'_1; R_s^*, α^* = corrected resolution and separation factors (Table II) ($R_s^* = R_s \times 1.67$; $\alpha^* = \alpha \times 1.12$)

Compound	t_R^1	k'_1	t_R^2	k'_2	α	α^*	R_s	R_s^*
1	11.33(−)	2.37	12.29(+)	2.66	1.12	1.30	0.66	1.10
2	7.99	1.38	8.46	1.52	1.10	1.28	0.43	0.71
3	11.29	2.36	13.07	2.89	1.22	1.42	0.64	1.06
4	10.24	2.05	12.12	2.61	1.27	1.47	0.58	0.96
5	8.36	1.49	8.79	1.62	1.08	1.25	0.50	0.83
6	10.34	2.08	10.82	2.22	1.08	1.25	0.53	0.88
7	8.01	1.38	8.49	1.53	1.12	1.30	0.33	0.55
8	11.01	2.28	11.62	2.46	1.07	1.24	0.54	0.90
9	27.28	7.12	30.66	8.13	1.14	1.32	0.57	0.95
10	10.80	2.21	12.29	2.66	1.20	1.39	1.03	1.76
11	10.97	2.26	13.27	2.95	1.31	1.52	0.96	1.61
12	7.85	1.34	8.29	1.47	1.09	1.26	0.61	1.01
13	10.66	2.17	11.51	2.43	1.12	1.30	0.76	1.26
14	13.01(−)	2.87	15.28(+)	3.55	1.23	1.43	0.78	1.29
15	12.34	2.67	15.12	3.50	1.31	1.52	0.83	1.38
16	12.81	2.81	17.85	4.31	1.53	1.77	0.55	0.91

the pterocarpan derivatives determined by us previously [14], we checked the activity of our column by measuring the separation values (α) of the unsubstituted pterocarpan and of maackiain, to establish whether or not the separation efficiency of the column had considerably changed.

A significant decrease in the resolution factor (R_s) was detected in both cases (Table II); this must have been the result of the decreased activity of the column (by about 40%), which was used for 150 h at room temperature. The most likely explanation for this decline is the solvolysis of PTrMA by methanol. It is also interesting to note that the decrease in the retention time (t_R) of the more strongly retained enantiomer with 6a*R*,11a*R* absolute configuration was double (without a considerable change of its capacity factor) that of the less retained one. We therefore introduced corrected resolution and separation factors, R_s^* and α^*, which were calculated using factors derived from the change in the separation efficiency of maackiain and **18** (Table I).

Since isoflavanone **1** could be just baseline resolved ($\alpha^* = 1.30$), the intensity of the π–π type non-polar interaction between the aromatic parts of the substrate and the pendant trityl groups of the chiral stationary phase seemed to be remarkably smaller than in case of **18** ($\alpha = 2.44$). Correlation of the configuration with the elution order revealed that the more strongly retained enantiomer [3*R*-(+)-**1**, t_R = 12.9 min] was homochiral at C-3 with the 6a*R*,11a*R*-configurated pterocarpan.

Since the chiral recognition mechanism of PTrMA is not yet understood in detail [19], it appeared to be of great interest to study the influence of the substitution pattern of a molecule on chiral recognition.

The presence of a hydroxy group at C-7 had a less significant influence (**2**, $\alpha^* = 1.28$) on the chiral recognition process than in the pterocarpan series (*cf.* **19**, $\alpha = 1.35$). The lowest enantioselectivity was observed for the 5,7-dihydroxylated isoflavanone (**7**, $R_s^* = 0.55$). Reducing the polar character of the molecule by etherification of the hydroxy group(s) improved the enantiomeric separation (R_s^* values: **2** < **5** < **4** < **3**, **7** < **8** < **9** and **12** < **13**) but not as efficiently as in the case of pterocarpans. The type of protecting groups caused different retention behaviours. Replacing the hydroxy group by a methoxy, isopropyloxy or methoxymethoxy group increased the retention time, which averaged up to *ca.* 2.7 and 3.5 min for the first- and second-eluted enantiomers, respectively. The retention times of these compounds (**3–6**, **8**, **13**) corresponded quite well to those of unsubstituted isoflavanone (**1**). In the case of **9**, a significant increase in retention time was observed for both enantiomers (t_R = 27.28 and 30.66 min) as a result of the presence of benzyloxy groups at C-5 and C-7, but the separation and the resolution factor ($\alpha^* = 1.32$, $R_s^* = 0.95$) were similar to those found for **1** ($\alpha^* = 1.30$, $R_s^* = 1.10$). These data clearly indicated that π–π interaction plays an important role in the separation, but this structural feature does not make any contribution to chiral recognition.

The π-electron density of the A- and B-rings of the isoflavanones seems to be very important for the recognition process, although a general rule cannot be derived from the results obtained so far.

The presence of a methylenedioxy group in ring B increased the enantiomeric separation (R_s^* for **2** < **12**, $\Delta R_s^* = 0.30$). The same tendency with a significantly larger effect was observed in the series of

TABLE II

DETAILED CHROMATOGRAPHIC DATA FOR EXPERIMENTS REPEATED BECAUSE OF THE OBSERVED CHANGE IN COLUMN ACTIVITY

(a) *Maackiain*
t_R (−) = 8.95 min, t_R (+) = 8.01 min; k' (−) = 2.01, k' (+) = 1.59; α = 1.26; R_s = 1.13

(b) ***18***
t_R (−) = 18.55 min, t_R (+) = 10.10 min; k' (−) = 5.20, k' (+) = 2.47; α = 2.10; R_s = 2.84

Changes in resolution values resulting from the decrease in the column activity in the case of:

(a) *Maackiain*
R_s = 2.20 [14] − 1.13 = 1.07 (49%)
Correction factor for R_s = 2.20/1.13 = 1.94
α = 1.35 [14] − 1.26 = 0.09 (7%)
Correction factor for α = 1.35/1.26 = 1.07

(b) ***18***
R_s = 4.00 [14] − 2.84 = 1.16 (29%)
Correction factor for R_s = 4/2.84 = 1.40
R_s^* = corrected resolution factor
= $R_s \times [(1.40 + 1.94)/2] = R_s \times 1.67$
α = 2.44 [14] − 2.10 = 0.34 (14%)
Correction factor for α = 2.44/2.10 = 1.16
α^* = corrected separation factor
= $\alpha \times [(1.15 + 1.07)/2] = \alpha \times 1.12$

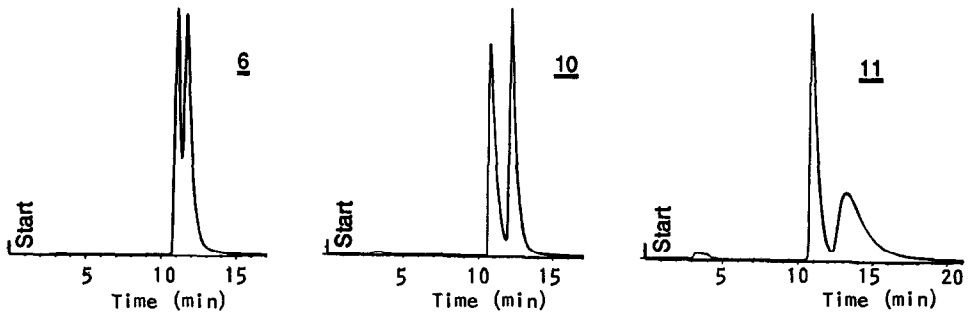


Fig. 3. Enantiomeric separation of **6**, **10** and **11** on Chiralpack OT(+) (Daicel). Mobile phase, methanol; flow-rate, 0.5 ml/min.

pterocarpans [R_s for 3-hydroxypterocarpan (**19**) < maackiain, ΔR_s = 1.20]. Electron-donating groups in ring A, *e.g.* methyl at C-8 (**10**) or methoxy at C-6 (**11**), also improved the separation (R_s^* values **6** < **11** < **10**). Moreover, the methoxy group changed the chromatographic profile strikingly, as illustrated in Fig. 3. The reason for this behaviour is still not known.

In order to obtain more information on the recognition mechanism of isoflavanones on PTrMA-coated silica gel, we also tested the resolution of some closely related compounds (**14–16**). Comparison of the separation of **1** with that of **14** (α^* = 1.30 and 1.43, respectively; R_s^* = 1.10 and 1.29, respectively) indicated that the introduction into the isoflavanoid skeleton of a carbonyl group possessing a large dipole moment disturbed chiral recognition. It is remarkable that the polarity of the mobile phase plays an important role in the separation process. Using *n*-hexane as the mobile phase at a flow-rate of 1 ml/min, the isoflavan **14** could be just baseline resolved (R_s^* = 1.14), and none of the examined isoflavanones were separable.

The homoisoflavanone **15** could be better resolved on PTrMA with methanol at a flow-rate of 0.5 ml/min (α^* = 1.52) than the corresponding isoflavanone **6** (α^* = 1.25) or the unsubstituted compound (**1**, α^* = 1.30). Substitution of the oxygen in ring C by CH_2 (**16**) gave a similar chromatographic profile as shown for **11** in Fig. 3, but the peak shape of its better-retained enantiomer was even more asymmetric, thus reducing the resolution factor significantly (R_s^* = 0.91).

In conclusion, PTrMA-coated silica gel is a suitable chiral stationary phase for resolving isoflavanones and homoisoflavanones. The enantioselectivity of the separation depends on different structural changes in the substituents and/or skeleton. Homoisoflavanones are better resolved than isoflavans or isoflavanones. Considerations on the chiral recognition mechanism based on computer-assisted molecular design will be published in a separate paper.

REFERENCES

1 H. Grisebach, in J. W. Benett and A. Ciegle (Editors), *Secondary Metabolism and Differentiation in Fungi*, Marcel Dekker, New York, 1983, pp. 377–428.
2 J. C. Ingham, *Phytopathol. Z.*, 87 (1976) 353.
3 P. M. Dewick, *Phytochemistry*, 16 (1977) 93.
4 T. P. Denny and A. D. van Etten, *Physiol. Plant Pathol.*, 19 (1981) 419.
5 V. J. Higgins, *Physiol. Plant Pathol.*, 2 (1972) 289.
6 P. M. Dewick and D. Ward, *J. Chem. Soc., Chem. Commun.*, (1977) 338.
7 W. Bless and W. Barz, *FEBS Lett.*, 235 (1988) 47.
8 D. Schlieger, K. Tiemann and W. Barz, *Phytochemistry*, 29 (1990) 1519.
9 H. Suginome, *Bull. Chem. Soc. Jpn.*, 39 (1966) 1544.
10 M. A. Fitzgerald, P. J. M. Gunning and D. M. X. Donnelly, *J. Chem. Soc. Perkin Trans. 1*, (1976) 186.
11 M. Kamatsu, I. Yokoe and Y. Shirataki, *Chem. Pharm. Bull.*, 26 (1978) 3863.
12 G. Delle Monache, F. Delle Monache, G. B. Marini-Bettolo, M. M. Albuquerque, J. F. de Mello and O. G. de Lima, *Gazz. Chim. Ital.*, 107 (1977) 189.
13 A. J. Kalra, M. Krishnamurti and M. Nath, *Indian J. Chem.*, 15B (1977) 1084.
14 A. S. Dreiding, *Helv. Chim. Acta*, 42 (1959) 1339.
15 S. Antus, R. Bauer, Á. Gottsegen and H. Wagner, *J. Chromatogr.*, 508 (1990) 212.
16 S. Antus, Á. Gottsegen, M. Nógrádi, *Synthesis*, (1981) 574.
17 S. Antus, E. Baitz-Gács, J. Cseri, Á. Gottsegen, O. Seligmann and H. Wagner, in preparation.
18 E. J. Corey and R. B. Mitra, *J. Am. Chem. Soc.*, 84 (1962) 2938.
19 A. M. Krstulovic (Editor), *Chiral Separations by HPLC*, Vol. 12, Wiley, New York, 1989, p. 316.