1-1-2003

# Cyclodextrin-based liquid chromatographic enantiomeric separation of chiral dihydrofurocoumarins and dihydrofuroflavones, emerging classes of medicinal compounds

Douglas David Schumacher
*Iowa State University*

# Cyclodextrin-based liquid chromatographic enantiomeric separation of chiral dihydrofurocoumarins and dihydrofuroflavones, emerging classes of medicinal compounds

by

**Douglas David Schumacher**

A thesis submitted of the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Analytical Chemistry

Program of Study Committee:
Daniel W. Armstrong, Major Professor
Shang-Yi Lin
Scott Chumbley

Iowa State University

Ames, Iowa

2003

Graduate College
Iowa State University

This is to certify that the master's thesis of

Douglas David Schumacher

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

# TABLE OF CONTENTS

## ABSTRACT

A set of 28 racemic dihydrofurocoumarins and 13 dihydrofuroflavones in which the stereogenic center is located in the furan ring have been synthesized. Currently no effective asymmetric synthesis for these classes of compounds exists, although they are produced naturally by many plant species. Their diverse medicinal properties are being investigated in several laboratories. The enantioselective separation of these compounds by three native and six derivatized cyclodextrins has been evaluated in the reversed phase mode, the polar organic mode, and normal phase mode. Overall, 20 of the 28 dihydrofurocoumarin and 9 of the 13 dihydrofuroflavone analytes were baseline resolved ($R_s > 1.5$) on at least one of the cyclodextrin-based chiral stationary phases. The hydroxypropyl-β-cyclodextrin (Cyclobond I RSP) is the most effective chiral stationary phase (CSP) for the enantioseparations of these compounds; baseline resolving 16 and 7 of the dihydrofurocoumarin and dihydrofuroflavone analytes respectively. The 2,3-dimethyl-β-cyclodextrin (Cyclobond I DM) also performed well, separating 18 dihydrofurocoumarin and 5 dihydrofuroflavone samples respectively. The acetyl-β-cyclodextrin (Cyclobond I AC) baseline resolved 18 of the dihydrofurocoumarin samples, however, no dihydrofuroflavones were separated on this CSP. The aromatic derivatized β-cyclodextrins are only marginally effective at separating the enantiomers of these compounds in the reversed phase mode. The native cyclodextrins showed no enantioselectivity for either class of compound in the reversed phase mode. The polar organic mode and the normal phase mode have also been evaluated with these CSPs, but no enantioseparations were observed.

# INTRODUCTION

In the search for medicinally important compounds, scientists have discovered several new classes of compounds. Of these, the dihydrofurocoumarin (Figure 1) and dihydrofuroflavone (Figure 2) derivatives have shown a great deal of promise as potential drug candidates. Both substituted dihydrofurocoumarins and substituted dihydrofuroflavones have been found in several plant species [1-11].

Both of these classes of compounds exhibit a variety of important biological effects. For dihydrofurocoumarins, the most significant of these properties are their photosensitizing and mutagenic activities [12-20], especially their ability to halt DNA replication by forming adducts with DNA nucleotides [17,18]. Dihydrofuroflavones are most useful for their antioxidant properties [21-30], particularly, their ability to inhibit the oxidative damage of DNA [22].

It is well know that the biological activity of each enantiomer of a chiral compound can vary greatly. Consequently, it has become standard practice to assess the biological activity of each enantiomer of a chiral molecule and market new products as single isomers [31]. To date, there has been no investigation of the biological activity of the individual enantiomers of chiral dihydrofuroflavones and very little investigation into the biological activity of chiral dihydrofurocoumarins [2,32]. Additionally, the development of asymmetric syntheses for these classes of compounds has been limited [33, 34]. Similarly, there have been no methods published in the literature pertaining to the enantioseparation of these classes of compounds. As such, methods must be developed to obtain both enantiomers of these compounds in their pure form and to determine the biological activity of each.

Recently, efforts by Rozhkov have generated chiral dihydrofurocoumarins [35] and chiral dihydrofuroflavones [36] by the palladium-catalyzed annulation of 1,3-dienes by *o*-iodo-umbelliferones and *o*-iodoacetoxyflavonoids respectively. Substituents on the dihydrofuran portion of the heterocycle create a stereogenic center in the furan ring of each (Fig. 1c and 2c).

Cyclodextrin based chiral stationary phases (CSPs) have been shown to be broadly applicable in their ability to separate enantiomers of a wide variety of compounds [37,38]. They are quite successful at resolving the enantiomers of chiral molecules with aromatic substituents [39-44]. Furthermore, it has been shown that cyclodextrins are useful in the analysis of various coumarin analogous such as warfarin, coumachlor, coumafuryl, phenprocoumon [42,45,46]. Additionally, β-cyclodextrin CSPs have been successful in resolving the enantiomers of several flavanone glycosides such as prunin, naringin, neohesperidin, and narirutin [47]. Consequently, cyclodextrin-based CSPs are a natural choice as CSPs for addressing the liquid chromatographic chiral separation of these types of compounds. The aim of this study is to evaluate the enantioselectivity of native and derivatized cyclodextrin based CSPs for these chiral dihydrofurocoumarins and chiral dihydrofuroflavones in all liquid chromatographic modes.

## Cyclodextrins

### History

The first documented use of Cyclodextrin-type molecules in a separation process was in 1959 by Cramer and Dietsche who evaluated their effectiveness as a selective cocrystalliziation agent for specific isomers of chiral compounds [48]. This technique, despite being successful at resolving some enantiomers of chiral compounds, is considered by many to be quite tedious and not universally applicable to chiral separations. In 1980, Armstrong used cyclodextrins as a chiral mobile phase additive in thin-layer chromatography [49]. The earliest success of binding a cyclodextrin moiety to silica was reported by both Fujimura and Kawaguchi in 1983 [50, 51]. These initial attempts, while successful at resolving the isomers of several aromatic compounds, were never commercialized due to the inherent instability of the linkage arm that bound the cyclodextrin selector to the silica support. The first successful cyclodextrin based CSP was developed by Armstrong in 1983 [52] and commercialized by Advanced Separation Technologies later that same year. This CSP overcame the limitations of predecessors by using a stable epoxide linkage to bind the

cyclodextrin to the silica support. This CSP has been used in the enantioseparation of many compounds such as amino acids [53], barbiturates [53], phenylacetic acid derivatives [53], metallocene enantiomers [54], and many other chiral aromatic compounds [55].

**Properties**

Cyclodextrins are chiral molecules consisting of cyclic oligosaccharides possessing a torodial shape (i.e., a hollow truncated cone) [56, 57]. These are produced either by the digestion of starch by the bacteria *Bacillus macerans* or by the enzymatic action of cyclodextrin transglycosylase. Cyclodextrins are produced in several sizes, but the most useful of these are the α- , β- , and γ- cyclodextrins which are composed of 6, 7, and 8 glucose units linked by α-1,4 bonds. The cavity sizes of these molecules are quite different. The cavity of an α-cyclodextrin can hold a molecule the size of a benzene ring, the β-cyclodextrin can accommodate a napthylene size molecule, and the γ-cyclodextrin can complex with 3-ringed systems such as anthracene or phenanthrene.

Another important structural feature of cyclodextrin molecules is the nature of the hyrdroxyl groups on the rims of the molecule. The large end or mouth of the cyclodextrin molecule is lined with secondary 2- and 3- hydroxyl groups and the narrow end or base is lined with primary 6- hydroxyls [58. 59]. These hydroxyls can facilitate chiral recognition by hydrogen bonding with analyte molecules. Additionally, the spatial orientation of these hydroxyl groups creates a hydrophobic interior cavity. The hydrophobic portion of analyte molecules can complex with or "include" into this cavity which is thought to be a major factor in the chiral recognition of molecules in aqueous solvents. The cyclodextrin's hydroxyl groups are also used to attach them to a stationary phase support via a linkage chain. Lastly, the cyclodextrin's hydroxyl groups can be functionalized with a variety of moieties to alter its enantioselectivity.

## Types of Cyclodextrin-based CSPs

There are several types of commercialized cyclodextrin CSPs. These can be subdivided into three major classes: native cyclodextrin CSPs, derivatized cyclodextrin CSPs, and aromatic derivatized CSPs. Each of these classes possesses different characteristics which are important to enantioseparations.

### Native Cyclodextrins

The most popular and useful of the native cyclodextrins is based on the β-cyclodextrin chiral selector. This selector has been shown to be suitable for the enantioseparation of many chiral compounds [42, 44, 55]. The α-cyclodextrin and γ-cyclodextrin CSPs are not as widely applicable as the β-cyclodextrin CSPs are, however, the α-cyclodextrin gave the only reported separation of monoterpene hydrocarbons such as α- and β-pinene [59].

### Derivatized and Aromatic Derivatized Cyclodextrins

There are several types of commercialized derivatized and aromatic derivatized cyclodextrin CSPs. All of these derivatives are based on the β-cyclodextrin chiral selector. The derivatized cyclodextrins CSPs are the acetylated-β-cyclodextrin (Cyclobond I AC), 2,3-dimethylated-β-cyclodextrin (Cyclobond I DM), and the hydroxypropyl-β-cyclodextrin (Cyclobond I RSP). The aromatic derivatized cyclodextrin CSPs include the napthylethylcarbamolate-β-cyclodextrin (Cyclobond I RN and SN) and the dimethylphenylcarbamate-β-cyclodextrin (Cyclobond I DMP). An overview of these CSPs is presented in Table 1.

All of these derivative groups are such that the effective size of the cyclodextrin cavity is expanded; allowing it to complex larger analyte molecules. Additionally, the aromatic derivatized cyclodextrins are the only cyclodextrin based CSPs useful in the normal phase mode [59].

## Mechanism of Chiral Recognition and Modes of Operation

For a chiral separation to occur there must be at least 3 differing yet simultaneous interactions about the stereogenic center. The types of interactions which are conducive to chiral recognition include $\pi$-$\pi$ interactions, steric interactions (repulsion), hydrophobic interactions, hydrogen bonding, dipole stacking, and electrostatic interactions. As with any separation method, it is possible to have interactions between the analyte and chiral selector which are not conducive to chiral recognition [68]. It is also possible to have two competitive interactions with opposite selectivities which may counteract any observed enantioselectivity [68].

There are three distinct modes of operation for the chromatographic enantioresolution of analytes on all cyclodextrin-based CSPs. These are the reversed phase mode, normal phase mode, and polar organic mode of operation; each of which has a unique mechanism for retention and chiral recognition. A summary of the properties of each mode is presented in Table 2.

## Thesis Organization

This study will investigate the enantioresolving power of cyclodextrin based CSPs for the separation of racemic mixtures of chiral dihydrofurocoumarins (Chapter One) and chiral dihydrofuroflavones (Chapter Two). All pertinent separation factors such as mobile phase composition, pH, buffer identity, and ionic strength are also investigated.

Previously, warfarin, coumachlor, coumafuryl, phenprocoumon, which are structurally related compounds to both dihydrofurocoumarins and dihydrofuroflavones, have been separated on the Cyclobond I DM [40,72], Cyclobond I [71], and Cyclobond I SN stationary phases [71]. Additionally, β-cyclodextrin CSPs have been used to separate the enantiomers of several flavanone glycosides such as prunin, naringin, neohesperidin, and narirutin [45]. This makes cyclodextrin based CSPs a natural choice for the enantioseparation of racemic mixtures of chiral dihydrofurocoumarins and dihydrofuroflavones .

## References

[1] R. C. Beiber, G. W. Ivie, E. H. Obertli, D. L. Holt, Food Chemistry and Toxicology, 21 (1983) 163-165.

[2] D. Guilet, J. J. Helesbeux, D. Seraphin, T. Sevenet, P. Richomme, J. Bruneton, Journal of Natural Products, 64 (2001) 563-568.

[3] C. A. J. Erdelmeier, B. Meier, O. Sticher, Journal of Chromatography, 346 (1985) 456-460.

[4] F. R. Stermitz, R. D. Thomas, Journal of Chromatography, 77 (1973) 431-433.

[5] M.G.L. Hertog, P.C Hollman, M.B. Katan, Journal of Agricultural Food Chemistry, 40 (1992) 2379-83.

[6] M.G.L. Hertog, P.C. Hollman, P. van de Putte, Journal of Agricultural Food Chemistry, 41 (1993) 1242-6.

[7] B.T. Ngadjui, E. Dongo, H. Tamboue, K. Fogue, B.M. Abegaz, Phytochemistry, 50 (1999) 1401-1406.

[8] B.T. Ngadjui, S.F. Kouam, E. Dongo, G.W.F. Kapche, B.M Abegaz, Phytochemistry, 55 (2000) 915-919.

[9] Wen-Kui Li, Pei-Gen Xiao, Ru-Yi Zhang, Phytochemistry, 38 (1995) 807-808.

[10] P. Maximo, A. Lourenco, S. Savluchinske Fieo, J.C. Roserio, Journal of Natural Products, 65 (2002) 175-178.

[11] D. Lee, K.P.L Bhat, H.H.S. Fong, N.R. Farnsworth, J.M. Pezzuto, A.D. Kinghorn, Journal of Natural Products, 64 (2001) 1286-1293.

[12] B. Bridges, G. Strauss, Nature 283 (1980) 523-524.

[13] E. L. Grant, R. C. von Borstel, M. J. Ashwood-Smith, Environmental Mutagenics, 1 (1979) 55-63.

[14] O. Schimmer, Planta Medica, 47 (1983) 79.

[15] B. R. Scott, M. K. Pathak, G. R. Mohn, Mutation Research 39 (1976) 29.

[16] M. A. Pathak, D. M. Kramer, T. B. Fitzpatrick, Sunlight and Man University of Tokyo Press (1973).

[17] L. Musajo, G. Rodighiero, (1972) In Gallo U. Santamaria, L. (eds): "Research Progress in Organic, Biological, and Medical Chemistry." Amsterdam: North Holland, vol 3, pp 155-182.

[18] L. Musajo, G. Rodighiero, Nature 190 (1961) 1109-1110.

[19] S. Y. Kang, K. Y. Lee, S. H. Sung, M. J. Park, Y. C. Kim, Journal of Natural Products, 64 (2001) 683-685.

[20] A. Jasinskas, N. Jasinskiene, J. P. Langmore, Biochimica and Biophysica Acta, 1397 (1998) 285-294.

[21] C. de-Whalley, S.M. Rankin, J.R.S Hoult, W. Jessup, D.S. Leake, Biochem. Pharmacology, 39 (1990) 1743-1750.

[22] M.K. Johnson, G. Loo, Mutation Research, 459 (2000) 211-218.

[23] R.L. Schleicher, C.A. Lamartiniere, M. Zheng, M. Zhang, Cancer Letters, 136 (1999) 195-201.

[24] V. Abalea, J. Cillard, M.P. Dubois, O. Sergent, P. Cillard, I. Morel, Free Radical Biology and Medicine, 26 (1999) 1457-1466.

[25] P. Pignatelli, F.M. Pulcinelli, A. Celestini, L. Lenti, A. Ghiselli, P. P. Gazzaniga, F. Violi, Am. Journal of Clinical. Nutrition, 72 (2000) 1150-1155.

[26] E.N. Frankel, J. Kanner, J.B. German, E. Parks, J.E. Kinsella, Lancet, 341 (1993) 454-457.

[27] G.T. McAnlis, J. Mc.Eneny, J. Pearce, I.S. Young, European Journal of Clinical Nutrition, 53 (1995) 92-96.

[28] B.J. Boersma, S. Barnes, M. Kirk, C.C. Wang, M. Smith, H. Kim, J. Xu, R. Patel, V.M. Darley-Usmar, Mutation Research, 480-481 (2001) 121-127.

[29] J.D. O'Reilly, A.I. Mallet, G.T. McAnlis, I.S. Young, B. Halliwell, T.A.B. Sanders, H Wiseman, American Journal of Clinical Nutrition, 73 (2001) 1040-1044.

[30] M. Chopra, P. Fitzsimons, J.J. Strain, D.I. Thurnham, A.N. Howard, Clinical Chemistry, 46 (2000) 1162-70.

[31] United States Food and Drug Administration, Chirality 4 (1992) 338-340.

[32] J. T. Trumble, J. G. Millar, Journal of Agricultural Food Chemistry, 44 (1996) 2859-2864.

[33] R. Tovar-Miranda, R. Cortes-Garcia, N. F. Santos-Sanches, P. Joseph-Nathan, Journal of Natural Products, 61 (1998) 1216-1220.

[34] V. Stanjek, M. Miksch, W. Boland, Tetrahedron 53 (1997) 17699-17710.

[35] R. V. Rozhkov, R. C. Larock, Organic Letters, 5(6) (2003) 797-800.

[36] R. V. Rozhkov, R. C. Larock, Organic Letters, (2003) In Press.

[37] D. W. Armstrong, W. DeMond, Journal of Chromatographic Science, 22 (1984) 411-415.

[38] D. W. Armstrong, T. J. Ward, R. D. Armstrong, T. E. Beesley, Science 232 (1986) 1132-1135.

[39] C. R. Mitchell, M. Desai, R. McCulla, W. Jenks, D. W. Armstrong, Chromatographia 56 (2002) 127-135.

[40] D. W. Armstrong, J. Zukowski, Journal of Chromatography A, 666 (1994) 445-448.

[41] D. W. Armstrong, T. J. Ward, A. Czech, B. P. Czech, R. A. Bartsch, Journal of Organic Chemistry, 50 (1985) 5556-5559.

[42] D. W. Armstrong, L. W. Chang, S. C. Chang, X. Wang, H. Ibrahim, G. R. Reid, T. E. Beesley, Journal of Liquid Chromatography and Related Technology, 20 (1997) 3279-3295.

[43] D.D. Schumacher, C.R. Mitchell, T.L Xiao, R.V. Rozhkov, R.C. Larock, D.W. Armstrong, Journal of Chromatography A, 1011 (2003) 37-47.

[44] D. W. Armstrong, W. DeMond, A. Alak, W. L. Hinze, T. E. Riehl H. K. Bui, Analytical Chemistry, 57 (1985) 234-237.

[45] N. A. Karnik, R. J. Prankerd, J. H. Perrin, Chirality 3 (1991) 124-128.

[46] A. Cepeda-Saez, P. Prognon, G. Mahuzier, J. Blais, Analytica Chimica Acta, 211 (1988) 333-337.

[47] M. Krause, R. Galensa, Journal of Chromatography, 588 (1991) 41-45.

[48] F. Cramer, W. Dietshe, Chemische Berichte, 92 (1959) 378-384.

[49] D. W. Armstrong, Journal of Liquid Chromatography, 3 (1980) 895-900.

[50] K. Fujimura, T. Veda, T. Ando, Analytical Chemistry, 55 (1983) 446-450.

[51] Y. Kawaguchi, M. Tanaka, M. Nakae, K. Funazo, T. Shono, Analytical Chemistry, 55 (1983) 1852-1857.

[52] D. W. Armstrong, W. DeMond, Journal of Chromatographic Science, 22 (1984) 411-415.

[53] W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak, T. J. Ward, Analytical Chemistry, 57 (1985) 237-242.

[54] D. W. Armstrong, W. DeMond, B. P. Czech, Analytical Chemistry, 57 (1985) 481-484.

[55] D. W. Armstrong, T. J. Ward, R. D. Armstong, T. E. Beesley, Science, 232 (1986) 1132-1135.

[56] M. L. Bender, M. Komiyama, Cyclodextrin Chemistry, Springer-Veriag – Berlin (1978).

[57] W. L. Hinze, Separation and Purification Methods, 10 (1981) 159-237.

[58] B. Gillet, D. J. Nicole, J. J. Delpuech, Tetrahedron Letters, 23 (1982) 65-68.

[59] D. W. Armstrong, A. M. Stalcup, M. L. Hilton, J. D. Duncan, J. R. Faulkner, S. C. Chang, Analytical Chemistry, 62 (1990) 1610-1615.

[60] D. W. Armstrong, J. Zukowski, Journal of Chromatography, 666 (1994) 445-448.

[61] S. C. Change, G. L. Reid, S. Chen, C. C. Chang, D. W. Armstrong, Trends in Analytical Chemistry, 12 (1993) 144-153.

[62] C. R. Mitchell, M. Desai, R. McCulla, W. Jenks, D. W. Armstrong, Chromatographia, 56 (2002) 127-135.

[63] D. D. Schumacher, C. R. Mitchell, T. L Xiao, R. V. Rozhkov, R. C. Larock, D. W. Armstrong, Journal of Chromatography A. 1011 (2003) 37-47.

[64] A. M. Stalcup, S. C. Chang, D. W. Armstrong, J. Pitha, Journal of Chromatography, 513 1990) 181-194.

[65] D. W. Armstrong, M. L. Hilton, L Coffin, LC-GC, 9 (1991) 646-652.

[66] D. W. Armstrong, G. L. Reid, M. L. Hilton, C. D, Chang, Environmental Pollution, 79 (1993) 51-58.

[67] D. D. Schumacher, R. V. Rozhkov, R. C. Larock, D. W. Armstrong, Journal of Chromatography A. (2003) In Press.

[68] R. E. Boehm, D. E. Martire, D. W. Armstrong, Analytical Chemistry, 60 (1998) 522-528.

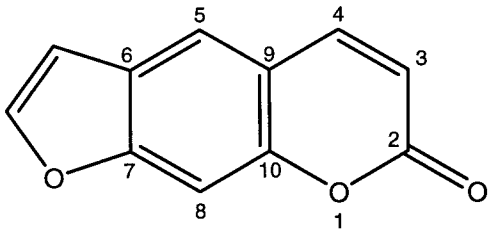
[69] C. A. Chang, Q. Wu, M. P. Eastman, Journal of Chromatography, 371 (1986) 269-282.

[70] S. Li, W. C. Purdy, Journal of Chromatography, 543 (1991) 105-112.

[71] S. C. Change, G. L. Reid, S. Chen, C. C. Chang, D. W. Armstrong, Trends in Analytical Chemistry, 12 (1993) 144-153.

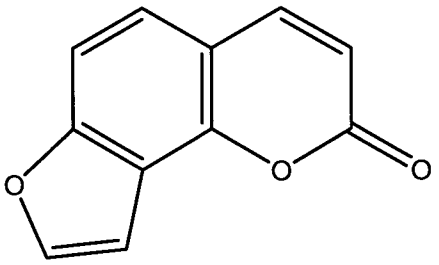
[72] N. A. Karnik, R. J. Prankerd, J. H. Perrin, Chirality 3 (1991) 124-128.

a)




psoralen

b)




angelicin

c)

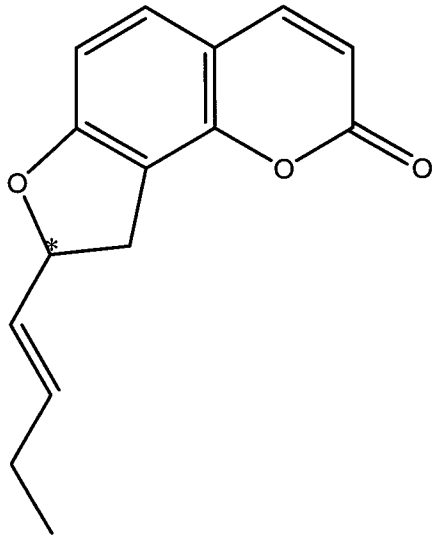



**Figure 1.** a) Psoralen type compounds are "linear" derivatives of coumarin where the furan ring is fixed to the 6,7-segment of the coumarin. The structure is numbered as the parent coumarin would be for consistency of comparisons in the discussion. b) Angelicin type compounds are derivatives of coumarin where the furan ring is fused to the 7,8-segment of the coumarin. c) A chiral, substituted angelicin analogue where the stereogenic center is denoted by an asterisk.

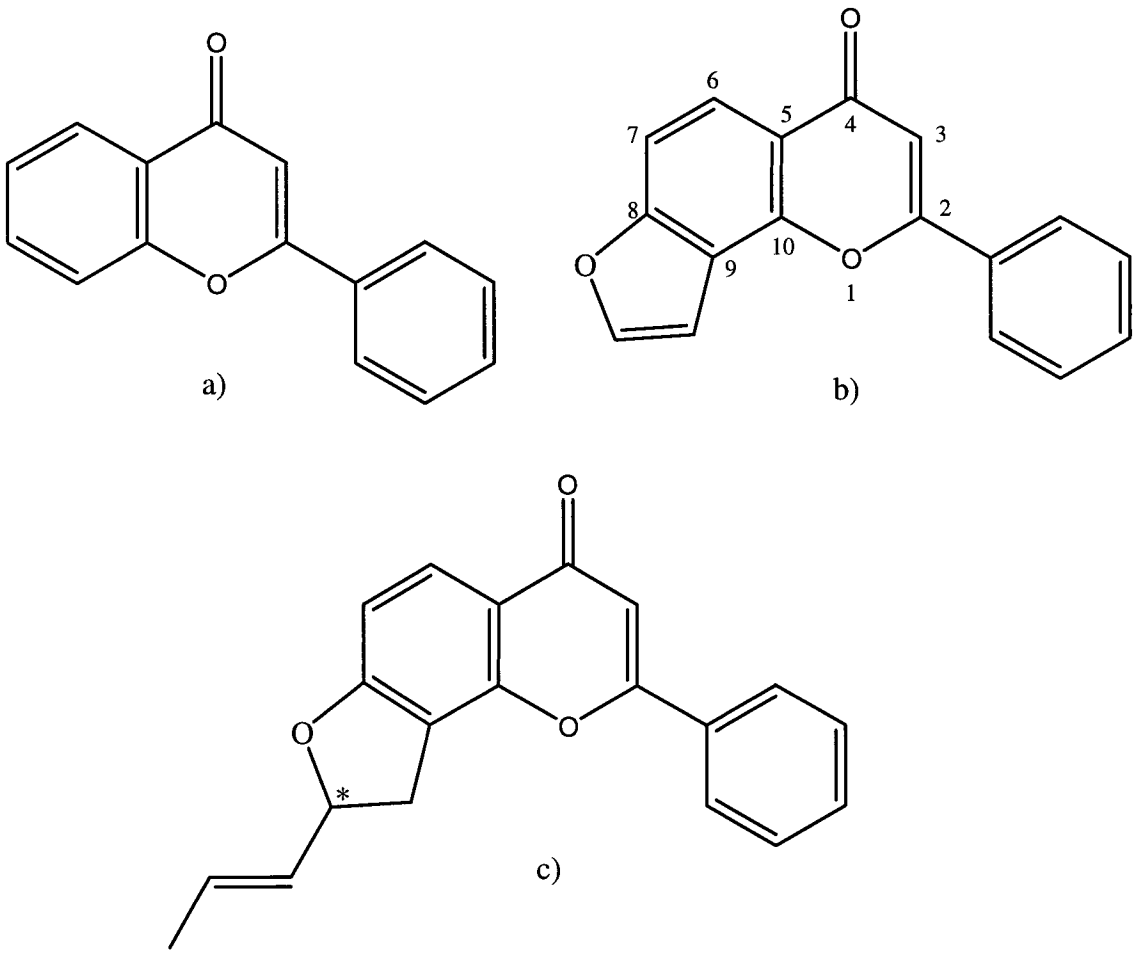
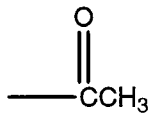
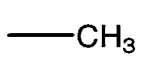
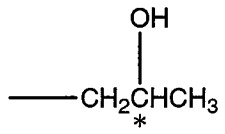
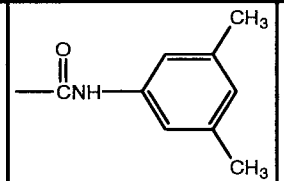
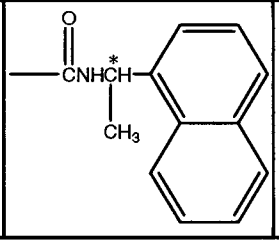



**Figure 2.** a) Flavone structure. b) Dihydrofuroflavones type compounds are derivatives of flavones where the furan ring is fused to the 7,8-segment of the flavone. The structure is numbered as the parent flavone for consistency of comparisons in the discussion. c) A chiral, substituted dihydrofuroflavone analogue where the stereogenic center is denoted by an asterisk.

**Table 1.** An Overview of Derivatized and Aromatic Derivatized Cyclodextrin CSPs.

| Column | Derivative Unit | Degree of Derivatization | [a] Modes of Operation | Possible Interactions | Types of Compounds Separated | Ref. |
|---|---|---|---|---|---|---|
| Cyclobond I AC |  | Complete | RP, PO | Hydrogen Bond Acceptor, Steric, [b] Inclusion Complexation | Pesticides Dihydrofurocoumarins | 51, 61 |
| Cyclobond I DM |  | All Secondary 2- and 3-hydroxyls | RP | Steric, [b] Inclusion Complexation | Sulfoxides Dihydrofurocoumarins Dihydrofuroflavones | 62, 63, 67 |
| Cyclobond I RSP |  | Approximately 7 units per cyclodextrin molecule | RP, PO | Hydrogen Bond Donating/Accepting Steric, [b] Inclusion Complexation | Sulfoxides Dihydrofurocoumarins Dihydrofuroflavones | 62-64, 67 |
| Cyclobond I DMP |  | Approximately 6 units per cyclodextrin molecule | RP, NP, PO | Hydrogen Bond Donating/Accepting Steric, p-p, Dipolar, [b] Inclusion Complexation | Sulfoxides | 62 |
| Cyclobond I RN/SN |  | Approximately 6 units per cyclodextrin molecule | RP, NP, PO | Hydrogen Bond Donating/Accepting Steric, p-p, Dipolar, [b] Inclusion Complexation | Pesticides Non-sterodial anti-inflammatorys | 59, 65, 66 |

[a] Modes of Operation: RP – Reversed Phase Mode, NP – Normal Phase Mode, PO – Polar Organic Mode

[b] Inclusion Complexation only occurs in the reversed phase mode of operation.

**Table 2.** Modes of Operation for Cyclodextrin based CSPs.

| Phase | Major Componet of Mobile Phase | Mobile Phase Modifiers | Additional Considerations | [a]Chiral Interactions | Ref. |
|---|---|---|---|---|---|
| **Reversed Phase Mode** | Water | Methanol, Ethanol, Isopropanol, Acetonitrile | pH, Buffer Identity, Ionic Strength, Molecule must contain a hydrophobic group, | **Inclusion Complexation**, Hydrogen Bonding, Steric Interactions, Dipolar Interactions | 53, 69, 70 |
| **Normal Phase Mode** | Hexane, Heptane | Isopropanol, Ethanol, Dichloromethane, Acetone | Molecule must contain an aromatic group | **π–π Interactions**, Steric Interactions, Hydrogen Bonding, Dipole Stacking | 32, 59 |
| **Polar Organic Mode** | Acetonitrile | Methanol, Triethylamine, Acetic Acid, | Molecule must contain at least two hydrogen bonding groups | **Hydrogen Bonding**, Dipolar Interactions, Steric Interactions | 71 |

[a] The main interaction responsible for chiral recognition is listed in **bold face** type

# CYCLODEXTRIN-BASED LIQUID CHROMATOGRAPHIC ENANTIOMERIC SEPARATION OF CHIRAL DIHYDROFUROCOUMARINS, AN EMERGING CLASS OF MEDICINAL COMPOUNDS

A paper published in the Journal of Chromatography A

Douglas D. Schumacher[1, 2], Clifford R. Mitchell[3], Tom L. Xiao[3], Roman V. Rozhkov[3], Richard C. Larock[3], and Daniel W. Armstrong[1]

## Abstract

A set of 28 racemic dihydrofurocoumarins in which the stereogenic center is located in the furan ring have been synthesized. Currently no effective asymmetric synthesis of this class of compounds exists, although their enantiomers are produced biologically by certain plants. Their diverse medicinal properties are being investigated in several laboratories. The enantioselective separation of these dihydrofurocoumarins by three native and six derivatized cyclodextrins has been evaluated in the reversed phase mode, the polar organic mode, and normal phase mode. The hydroxypropyl-β-cyclodextrin is the most effective chiral stationary phase (CSP) at separating the dihydrofurocoumarins into enantiomers, showing some enantioselectivity for 22 dihydrofurocoumarins, and baseline resolving 16 of the 28 compounds in the reversed phase mode. The acetyl-β-cyclodextrin and 2,3-dimethyl-β-cyclodextrin also showed enantioselectivity for a large number (18 and 17 respectively) of dihydrofurocoumarins in the reversed phase mode. The native cyclodextrins are ineffective and the aromatic derivatized β-cyclodextrins are only marginally effective at separating the

---

[1] Graduate Student and Major Professor, respectively, Department of Chemistry Iowa State University.

[2] Primary researcher and author.

[3] Author in correspondence.

furocoumarin enantiomers in the reversed phase mode. The polar organic mode and the normal phase mode have also been evaluated with these CSPs, but no enantioseparations were observed.

## Introduction

Over the last several years, furocoumarins have received considerable attention from chemists, biologists, and pharmacologists. Two general classes of furocoumarins are the psoralens and angelicins (Fig. 1). Both of these classes of compounds contain the parent coumarin structure fused to a dihydrofuran ring. Furocoumarins are found many places in nature, most often in plants. Different substituted furocoumarins have been found in celery [1], bark extracts [2], citrus oil [3], and culinary herbs (parsley [4], dill, fennel, and cumin).

Furocoumarins are known to exhibit a variety of biological effects. Most significant are their photosensitizing and mutagenic activities [5-8]. Ancient Egyptians used psoralens in the form of plant extracts for the treatment of skin disorders [9]. In more recent times, furocoumarins have been used for the treatment of psoriasis and vitiligo (skin de-pigmentation). Naturally occurring psoralen, bergapten, and xanthotoxin were found to be most active against skin diseases and were used in PUVA therapy (Psoralen-UltraViolet A). Upon exposure to long wavelength UV light (320-380 nm) furocoumarins form adducts with DNA nucleotides [10,11]. These adducts prevent the proliferation of cells from damaged or diseased tissues by halting DNA replication, which disrupts cellular division. While both mono- and di-adducts have therapeutic effects, inter-strand cross-linked adducts are primarily responsible for un-repairable DNA damage and undesired mutagenic effects [6]. More recently, furocoumarins have been investigated for their ability to inhibit acetylcholinesterase [12], their cytotoxicity against KB cells [2] (a line of cancerous cells), and for distinguishing between active and inactive rRNA [13].

It is well know that the biological activity of enantiomeric compounds can vary greatly. Consequently, it has become standard practice to assess the biological activity of each enantiomer of a chiral molecule and to produce drugs and food products mainly as

single enantiomers [14]. Only recently has there been any investigation into the biological activity of chiral dihydrofurocoumarins [2,15]. To date, the development of asymmetric syntheses of chiral dihydrofurocoumarins has been limited [16,17] and there have been no methods published in the literature pertaining to the enantioseparation of chiral dihydrofurocoumarins. As such, methods must be developed to obtain both enantiomers in their pure form and to determine the activity of each.

Cyclodextrin based chiral stationary phases (CSPs) have been shown to be broadly applicable in their ability to separate enantiomers of a wide variety of compounds [18,19]. They are quite successful at resolving the enantiomers of chiral molecules with aromatic substituents [20-24]. Furthermore, it has been shown that cyclodextrins are useful in the analysis of various coumarin derivatives, as a CSP for the enantioseparation of warfarin, coumachlor, coumafuryl, phenprocoumon [23,25], and as post column fluorescence enhancing reagents for psoralen and phenprocoumon [25,26]. Consequently, cyclodextrin-based CSPs are a natural choice as CSPs for addressing the liquid chromatographic chiral separation of these compounds.

Recent efforts by Rozhkov et. al. [27] have generated chiral dihydrofurocoumarins by the palladium-catalyzed annulation of 1,3-dienes by *o*-iodo-umbelliferones. Substituents on the dihydrofuran portion of the heterocycle create a stereogenic center (Fig. 1c). The aim of this work is to evaluate the enantioselectivity of native and derivatized cyclodextrin based CSPs for these chiral dihydrofurocoumarins. Both substituted psoralens and substituted angelicins are examined in different chromatographic modes.

## Experimental

### Materials

The CSPs were obtained from Advanced Separation Technologies (Whippany, NJ, USA). All stationary phases used consisted of the chiral selector bonded to 5 μm spherical silica gel. The chiral selectors used are the underivitized cyclodextrins and the derivatized β-cyclodextrins, which are illustrated in Fig. 2. The dimensions of the columns are $250 \times 4.6$

mm. The triethylamine, methanol, acetonitrile, 2-propanol, and hexane used were HPLC grade from Fisher (Fairlawn, NJ, USA). Sodium chloride and acetic acid were ACS certified grade from Fisher. All substituted dihydrofurocoumarin were prepared as outlined previously [27].

## Equipment

The HPLC system used consisted of a quaternary pump, an auto sampler, a UV VWD detector (1050, Hewlett Packard, Palo Alto, CA, USA), and an integrator (3395, Hewlett Packard). Mobile phases were degassed by ultra-sonication under vacuum for 10 minutes. UV detection was carried out at 220 nm. All separations were carried out at room temperature (~23°C).

## Column Evaluation

The performance of each stationary phase was evaluated in the reverse phase mode using acetonitrile/water and methanol/water mobile phases. The aromatic derivatized CSPs, Cyclobond DMP, RN, and SN, were also evaluated in the normal phase mode (isopropanol/hexane) and in the polar organic mode (100% acetonitrile). The composition of the mobile phase was optimized for resolving the enantiomers of each compound at a flow rate of 1.0 mL $min^{-1}$.

## Calculations

Dead times ($t_M$) were estimated using the refractive index solvent peak on each CSP. Retention factors ($k$) were calculated using the equation $k = (t_r - t_M)/t_M$. Enantioselectivity ($\alpha$) was calculated using the equation $\alpha = k_2/k_1$. Resolution factors ($R_s$) was calculated using the equation $R_s=2 \times(t_r2 - t_r1) / (w_1 + w_2)$, where $t_r2$ and $t_r1$ are the retention times of the second and first enantiomers respectively and $w_1$ and $w_2$ are the base peak widths of the corresponding peaks.

## Results and Discussion

A series of 28 racemates, including 7 substituted psoralen derivatives, 14 substituted angelicin derivatives, 5 substituted dihydrofurocoumarins, and 2 substituted coumarins were evaluated on nine different cyclodextrin based CSPs in the reversed phase mode (see Table I for structures and separation data). Fig. 3 is a summary of the performance of each CSP in the reversed phase mode. Clearly the best CSP for these chiral dihydrofurocoumarins utilizes hydroxypropyl-β-cyclodextrin as the chiral selector (Cyclobond I RSP). The acetyl-β-cyclodextrin (Cyclobond I AC) and 2,3-dimethyl-β-cyclodextrin (Cyclobond I DM) based CSPs were also able to resolve a large number of dihydrofurocoumarins. The remaining CSPs, native cyclodextrins and aromatic derivatized β-cyclodextrin, were either ineffective or showed enantioselectivity for a small number of the examined dihydrofurocoumarin compounds in the reversed phase mode. A partial separation of enantiomers is reported in Fig. 3 if there is an observable enantioselectivity ($\alpha > 1.02$) and a baseline separation of enantiomers is reported if the peak-to-peak resolution ($R_s$) exceeds 1.5.

The effect of mobile phase composition was also investigated. All 28 compounds were analyzed in the reversed phase mode with both acetonitrile/water and methanol/water mobile phases on all CSPs. Generally, comparable results for enantioselectivity and resolution were obtained with each solvent system; however, there were several cases where an acetonitrile/water mobile phase successfully separated enantiomers where the methanol/water mixture failed. This is thought to be due to hydrogen bonding of the methanol molecules to the hydroxyl groups on the cyclodextrin, which may interfere with the enantioselective complexation process. The effect of pH (4.00, 5.00, 6.00, 7.00, and unbuffered [pH = 6.20], 0.1% (*v/v*) triethylamine/acetic acid) and ionic strength (0 M, 0.10 M, 0.20, 0.30, 0.40, and 0.50 M NaCl) were also investigated. However, neither appreciably affected selectivity or resolution (data not shown). This is due to the fact that the dihydrofurocoumarins are neutral, hydrophobic compounds with no ionizable groups (see Fig. 1 and Table I).

**Cyclobond I RSP, AC, and DM Chiral Stationary Phases**

Table I summarizes the separation data for the most effective Cyclobond AC, DM, and RSP columns in the reversed phase mode of operation. The structure of each dihydrofurocoumarin and the optimal mobile phase compositions are given, as well as the values for *k*, *Rs*, and $\alpha$.

It is well known that cyclodextrin CSPs excel at enantioseparations where the analytes contain large aliphatic groups or multiple aromatic rings [20-24]. For example, the separation of angelicin derivatives 1, 2, and 3 clearly show that an increase in steric bulk about the stereogenic center improves the separation on all 3 of the non-aromatic derivatized β-cyclodextrin CSPs (Table I). On the hydroxypropyl-β-cyclodextrin CSP, the resolution of these compounds is enhanced (see Fig. 4). Compound 14 also shows that an excess of steric bulk can hinder a separation on some CSPs (Cyclobond I AC and DM) and enhance selectivity on others (Cyclobond I RSP).

Other examples of the importance of steric interactions near the chiral center are shown in the separation of compounds 9, 10, and 11 on these CSPs. While these molecules are structurally similar, the addition or removal of one methyl group alpha to or beta to the stereogenic center can greatly affect the observed enantioselectivity (see Fig. 5). The methyl groups create additional steric bulk near the chiral center, which enhances chiral recognition. Conversely, compounds 6, 8, and 12 have little steric bulk near the chiral center, leading to diminished enantioselectivity. Therefore, steric bulk must play a significant role in the selectivity of these types of compounds.

The enantioseparations of the dihydrofuroangelicin derivatives and their corresponding structural isomers (the dihydrofuropsoralen derivatives) is also of interest. While these pairs of analytes are quite similar, the more-linear psoralen derivatives are generally less well resolved than their angelicin derivative counterparts. This is the case for compounds 8 and 20, 9 and 24, and 13 and 25. The results for compounds 8 and 20, and 13 and 25 are shown in Fig. 6. The difference in enantioselectivity between dihydrofuroangelicin derivatives and dihydrofuropsoralen derivatives must be due to the

spatial orientation of the dihydrofuran group, which limits the rotational or reorientational ability of the analyte in the inclusion complex. It is also if interest to note that, when comparing dihydrofuroangelicin derivatives with the dihydrofuropsoralen derivative structural analogs, a separation of enantiomers is not achieved in the case of the dihydrofuropsoralen analytes. For example, compare compounds 7, 8, and 12, which are dihydrofuroangelicin derivatives, with compounds 21, 20, and 26 which are the corresponding dihydrofuropsoralen derivatives (which are not separated into enantiomers). There is only one case where a dihydropsoralen analogue is better resolved than its dihydroangelicin counterpart - compounds 6 vs. 22.

It was also observed that the orientation of the dihydrofuran oxygen in relation to the coumarin affects the enantiomeric separation. For example, compounds 17 and 18 are very similar in structure, as are compounds 15 and 16. Figure 7 is a comparison of the enantiomeric separation of compounds 17 and 18 (which differ only in the location of the oxygen heteroatom in the furan ring) on the Cyclobond I RSP. The best chiral selector for this class of compounds is the hydroxypropyl-β-cyclodextrin (Cyclobond I RSP), as all four of these analytes are baseline resolved ($Rs \geq 1.5$). Greater selectivity was observed when the dihydrofuran oxygen was alpha to position 5 on the coumarin for the Cyclobond I RSP (compounds 16 and 18) and alpha to position 6 for the Cyclobond I AC column (compounds 15 and 17).

Obviously the exact location of the fused dihydrofuran ring (on the parent coumarin) has a significant impact on the separation. This is further shown by comparing the results from compounds 1, 15, 16, and 19 on the Cyclobond I AC and RSP CSPs. The best orientation for enantioresolution on these CSPs is when the dihydrofuran moiety is fused to the 5 and 6 positions on the coumarins, as is the case for compounds 15 and 16.

**Other CSPs – Native and Aromatic Derivatized Cyclodextrins**

Other cyclodextrin-based CSPs were much less effective in separating enantiomers of these types of compounds in the reversed phase mode. These remaining CSPs can be divided

into two categories: aromatic derivatized cyclodextrin CSPs (Cyclobond I RN, SN and DMP) and native cyclodextrin CSPs (Cyclobond I, II, and III). The results of these analyses are presented in Table II. The aromatically derivatized cyclodextrins (Cyclobond I DMP, RN, and SN) are not as successful for this class of compounds. As only a limited number of separations were observed with the aromatically derivatized cyclodextrins, it is reasonable to conclude that an excess of aromatic steric bulk on the chiral selector is detrimental to the enantioseparation of most chiral dihydrofurocoumarins. The native cyclodextrins did not show any selectivity for any of the analytes investigated.

**Normal Phase and Polar Organic Modes**

The normal phase mode was investigated on all of the aromatically derivatized CSPs. The Cyclobond I RN, SN, and DMP columns were each evaluated with a 5/95 isopropanol/hexane mobile phase. All analytes were appreciably retained, but no enantioselectivity was observed. The polar organic mode was also investigated under the weakest condition (100% acetonitrile) where all compounds eluting at the dead time of the column.

**Mechanistic Observations**

The binding of a dihydrofurocoumarin analyte to a cyclodextrin CSP is a dynamic process. Both the furan portion and the lactone portion of a dihydrofurocoumarin molecule can enter the cyclodextrin cavity to form an inclusion complex in the reverse phase mode, but only one of the two inclusion complex orientations will produce the enantioselectivity which leads to the observed chiral separation. It is well established that, for a cyclodextrin to form an enantioselective diastereomeric complex, the substituents off of the stereogenic center of the analyte must be in close proximity to the secondary hydroxyls at the mouth of the cyclodextrin in order to achieve the necessary three-points of interaction [19,24,28]. If the furan portion of the molecule resides in the cavity of the cyclodextrin upon inclusion, the stereogenic center will be buried inside the cyclodextrin torus, not in close proximity to the

secondary hydroxyl groups (or the derivative groups on these hydroxyls) on the larger rim of the molecule. In this case, the substituents on or near the analyte's stereogenic center will be unable to interact with the porton of the chiral selector that is most responsible for chiral recognition. It is then reasonable to conclude that, for chiral recognition to occur, the lactone portion of the analyte molecule must occupy the cyclodextrin cavity and the furan portion is in close proximity to the mouth of the cyclodextrin cavity where the secondary hydroxyls and their substituents are located.

The size of these analytes (Table I and II) supports the contention that the same portion of these polycyclic analytes must protrude from the torus of the cyclodextrin cavity when an inclusion complex is formed. The hydroxylpropyl-β-cyclodextrin CSP and acetyl-β-cyclodextrin CSP (Cyclobond I RSP and AC) are very successful at resolving larger analytes where significant portions of the included molecule protrude from the cyclodextrin (23, 29), whereas native cyclodextrins are not. The hydroxylpropyl and acetyl groups of the derivatized cyclodextrins are also known to extend beyond the mouth of the cyclodextrin cavity (28) and are in a position to interact with both the dihydrofuran moiety and any substituents attached to the stereogenic center. This has previously been shown to be the most prominent interaction that leads to enantioselectivity in the cases when the hydroxylpropyl-β-cyclodextrin CSP is superior to the native β-cyclodextrin CSP [29]. Therefore, the additional interactions produced by these derivative groups are essential for chiral recognition. Taking into consideration the fact that native cyclodextrins CSPs are completely ineffective in separating these compounds, one must conclude that when the dihydrofurocoumarins form an enantioselective inclusion complex with a derivatized cyclodextrin in the reversed phase mode, their stereogenic center be located near the mouth of the cyclodextrin selector.

## Conclusions

The Cyclobond I AC, DM and RSP are the most effective cyclodextrin-based CSPs for resolving the enantiomers of chiral dihydrofurocoumarins in the reversed phase mode. This is due to the analyte complexing with these chiral selectors in such a way that the substituents off of the dihydrofurocoumarin stereogenic center interact with the derivative moieties on the cyclodextrin molecule. The presence of steric bulk about the analytes chiral center greatly enhances the chiral recognition of these enantiomers. The orientation of the furan oxygen as well as the spatial placement of the dihydrofuran moiety on the parent coumarin molecule both play a major role in the selectivity of the separation. Generally, the angelicin-type coumarins are better resolved than their psoralen analogues. The aromatic derivatized and native cyclodextrins are mostly ineffective at resolving these types of analytes. The normal phase and polar organic modes could not be used to separate any of these compounds into their enantiomers with these CSPs.

## Acknowledgements

Support of this work by the National Institutes of Health, NIH RO1 GM53825-06 is gratefully acknowledged.

R. V. Rozhkov and R. C. Larock also acknowledge the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research and Johnson Matthey Inc. and Kawaken Fine Chemicals Co., for donations of palladium acetate.

## References

[1] R. C. Beiber, G. W. Ivie, E. H. Obertli, D. L. Holt, Food Chem. Toxicol. 21 (1983) 163-165.

[2] D. Guilet, J. J. Helesbeux, D. Seraphin, T. Sevenet, P. Richomme, J. Bruneton, J. Nat. Prod. 64 (2001) 563-568.

[3] C. A. J. Erdelmeier, B. Meier, O. Sticher, J. Chromatogr. 346 (1985) 456-460.

[4] F. R. Stermitz, R. D. Thomas, J. Chromatogr. 77 (1973) 431-433.

[5] B. Bridges, G. Strauss, Nature 283 (1980) 523-524.

[6] E. L. Grant, R. C. von Borstel, M. J. Ashwood-Smith, Environ. Mutagen. 1 (1979) 55-63.

[7] O. Schimmer, Planta Med. 47 (1983) 79.

[8] B. R. Scott, M. K. Pathak, G. R. Mohn, Mutation Research 39 (1976) 29.

[9] M. A. Pathak, D. M. Kramer, T. B. Fitzpatrick, Sunlight and Man University of Tokyo Press (1973).

[10] L. Musajo, G. Rodighiero, (1972) In Gallo U. Santamaria, L. (eds): "Research Progress in Organic, Biological, and Medical Chemistry." Amsterdam: North Holland, vol 3, pp 155-182.

[11] L. Musajo, G. Rodighiero, Nature 190 (1961) 1109-1110.

[12] S. Y. Kang, K. Y. Lee, S. H. Sung, M. J. Park, Y. C. Kim, J. Nat. Prod. 64 (2001) 683-685.

[13] A. Jasinskas, N. Jasinskiene, J. P. Langmore, Biochim. Biophys. Acta 1397 (1998) 285-294.

[14] United States Food and Drug Administration, Chirality 4 (1992) 338-340.

[15] J. T. Trumble, J. G. Millar, J. Agric. Food. Chem. 44 (1996) 2859-2864.

[16] R. Tovar-Miranda, R. Cortes-Garcia, N. F. Santos-Sanches, P. Joseph-Nathan, J. Nat. Prod. 61 (1998) 1216-1220.

[17] V. Stanjek, M. Miksch, W. Boland, Tetrahedron 53 (1997) 17699-17710.

[18] D. W. Armstrong, W. DeMond, J. Chromatogr. Sci. 22 (1984) 411-415.

[19] D. W. Armstrong, T. J. Ward, R. D. Armstrong, T. E. Beesley, Science 232 (1986) 1132-1135.

[20] C. R. Mitchell, M. Desai, R. McCulla, W. Jenks, D. W. Armstrong, Chromatographia 56 (2002) 127-135.

[21] D. W. Armstrong, J. Zukowski, J. Chromatogr. A 666 (1994) 445-448.

[22] D. W. Armstrong, T. J. Ward, A. Czech, B. P. Czech, R. A. Bartsch, J. Org. Chem. 50 (1985) 5556-5559.

[23] D. W. Armstrong, L. W. Chang, S. C. Chang, X. Wang, H. Ibrahim, G. R. Reid, T. E. Beesley, J. Liq. Chromatogr. Related Technol. 20 (1997) 3279-3295.

[24] D. W. Armstrong, W. DeMond, B. P. Czech, Anal. Chem. 57 (1985) 481-484.

[25] N. A. Karnik, R. J. Prankerd, J. H. Perrin, Chirality 3 (1991) 124-128.

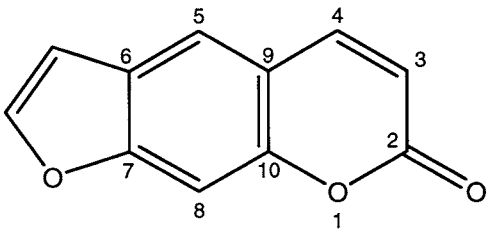
[26] A. Cepeda-Saez, P. Prognon, G. Mahuzier, J. Blais, Anal. Chim. Acta 211 (1988) 333-337.

[27] R. V. Rozhkov, R. C. Larock, Org. Lett. 5(6) (2003) 797-800.

[28] W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak, T. J. Ward, Anal. Chem. 57 (1985) 237-242.

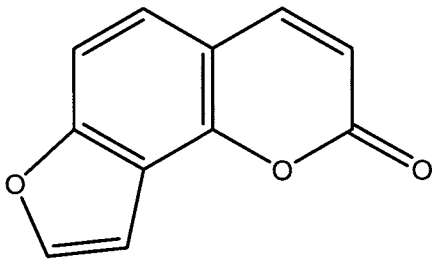
[29] A. M. Stalcup, S. C. Chang, D. W. Armstrong, J. Pitha, J. Chromatogr. 513 (1990) 181-194.

a)




psoralen

b)




angelicin

c)

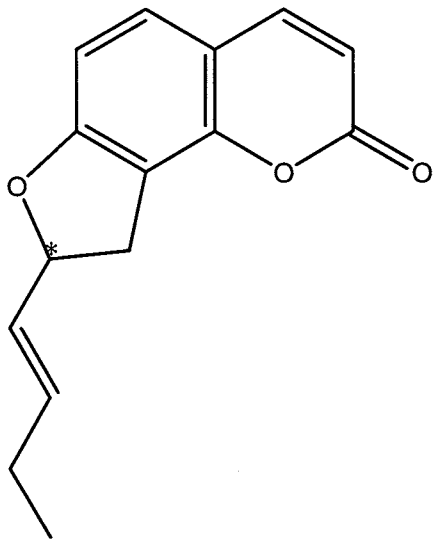



**Figure 1.** a) Psoralen type compounds are "linear" derivatives of coumarin where the furan ring is fixed to the 6,7-segment of the coumarin. The structure is numbered as the parent coumarin would be for consistency of comparisons in the discussion. b) Angelicin type compounds are derivatives of coumarin where the furan ring is fused to the 7,8-segment of the coumarin. c) A chiral, substituted angelicin analogue where the stereogenic center is denoted by an asterisk.

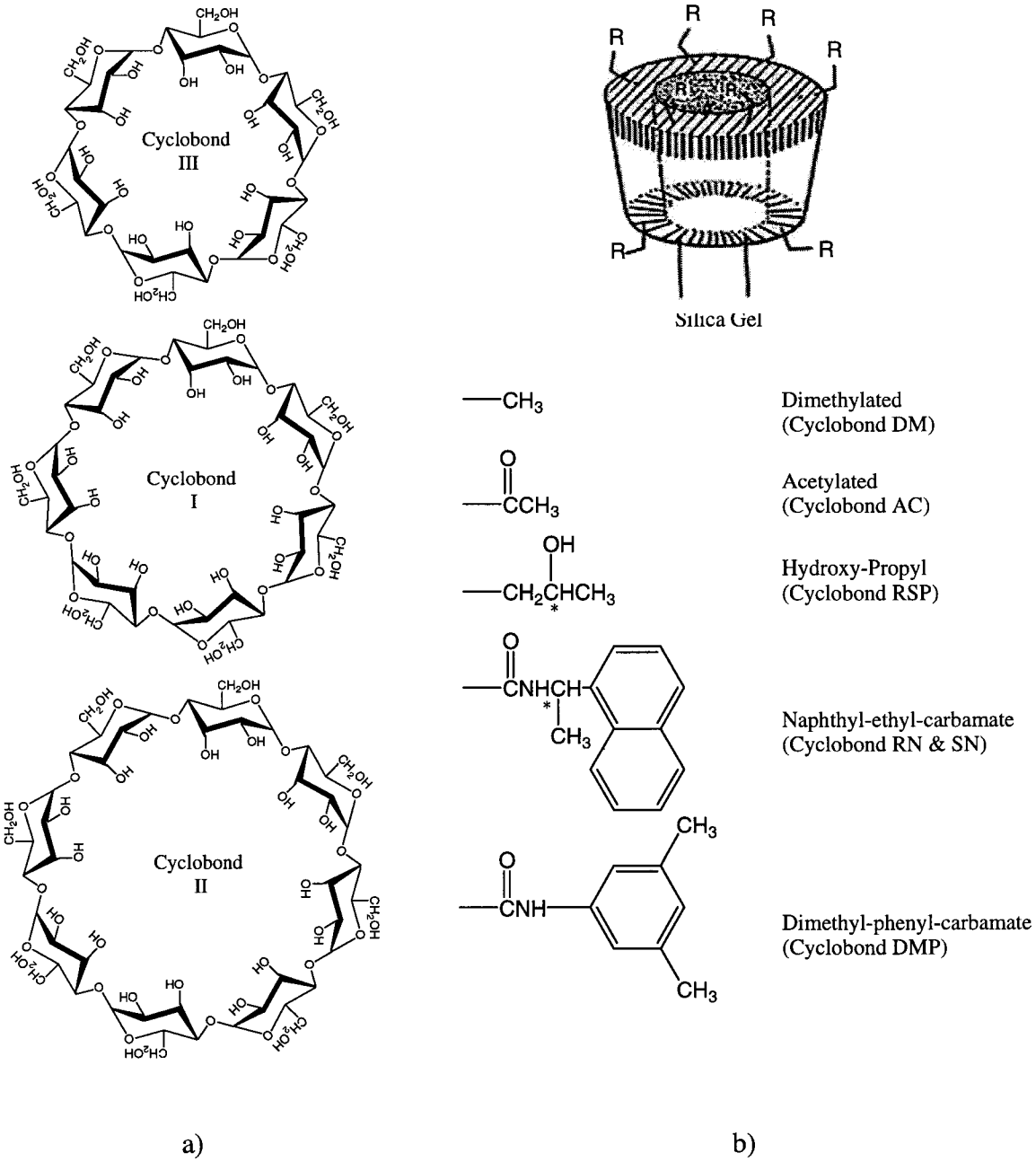



**Figure 2.** a) Native alpha, beta, and gamma cyclodextrins (i.e. Cyclobond III, I and II respectively). b) Types of derivatized cyclodextrins. An asterisk denotes the stereogenic center. Reproduced from Reference 27.

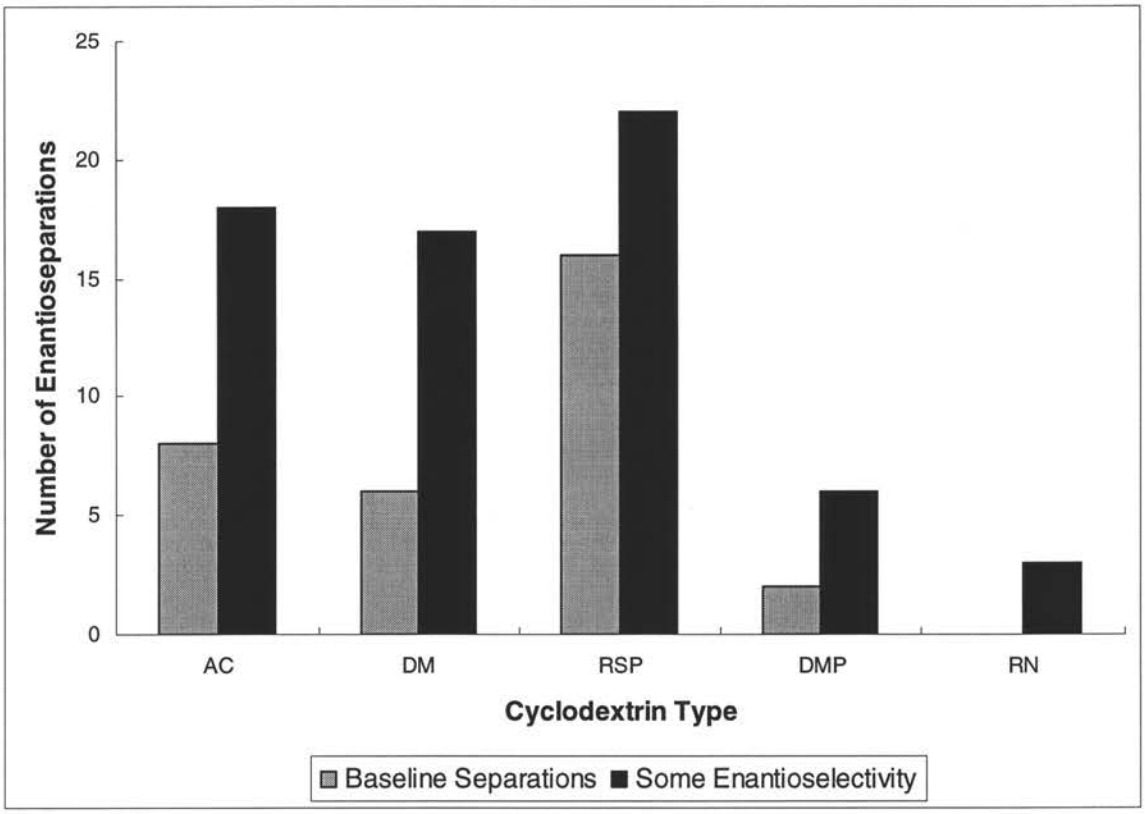



**Figure 3.** Number of separations in the reversed phase mode using cyclodextrin-based CSPs. The various types of cyclodextrins and their designated abbreviations are illustrated in Figure 1. Grey Bars: number of observable enantioselective separations, enantioselectivity, $\alpha$, > 1.02. Black Bars: number of baseline separations, enantioresolution, *Rs*, > 1.5.

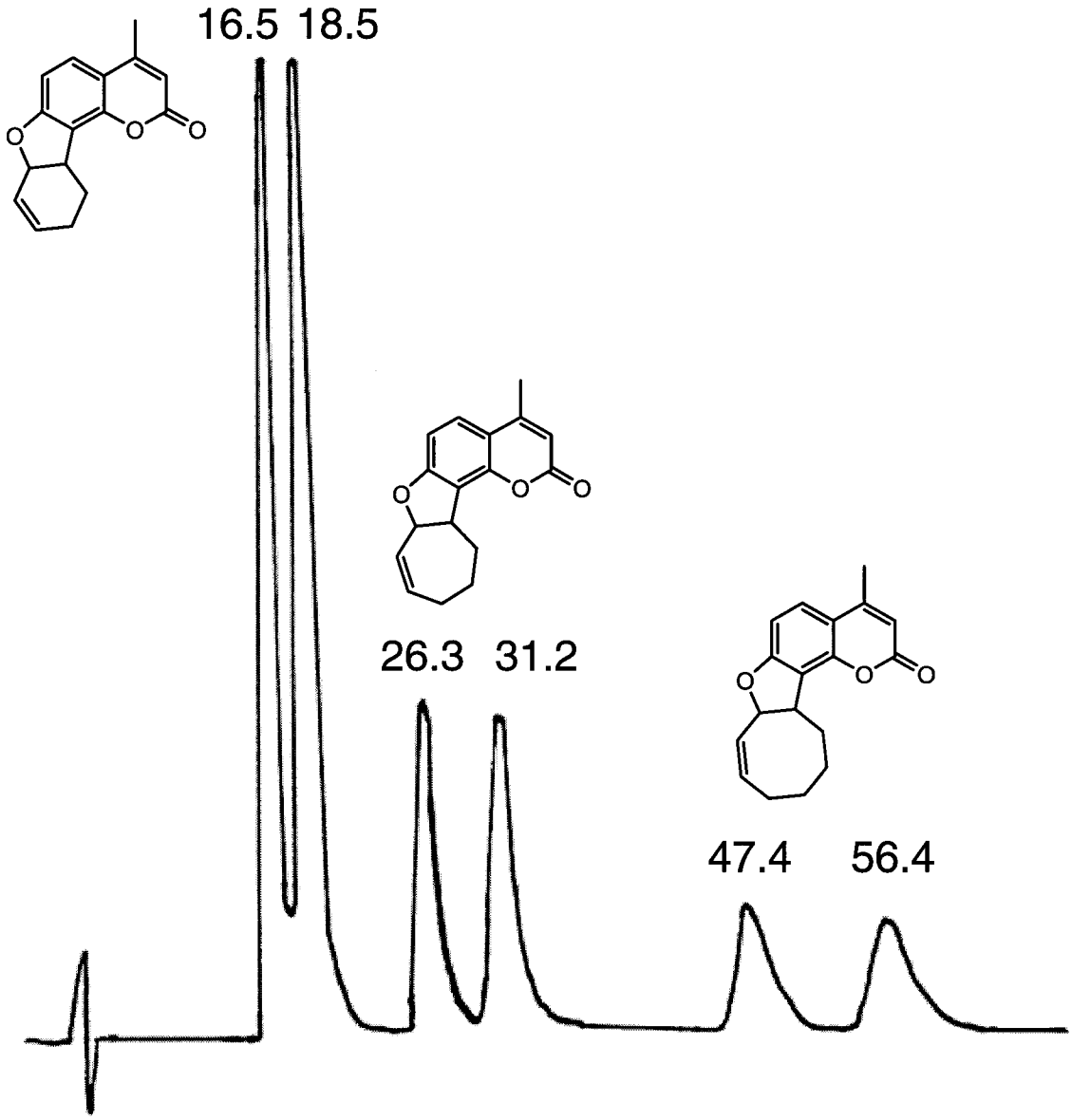



**Figure 4.** Enantioseparation of Dihydrofurocoumarins 1, 2, and 3 (in order of elution) on Cyclobond RSP. Mobile Phase 45/55 methanol/water.

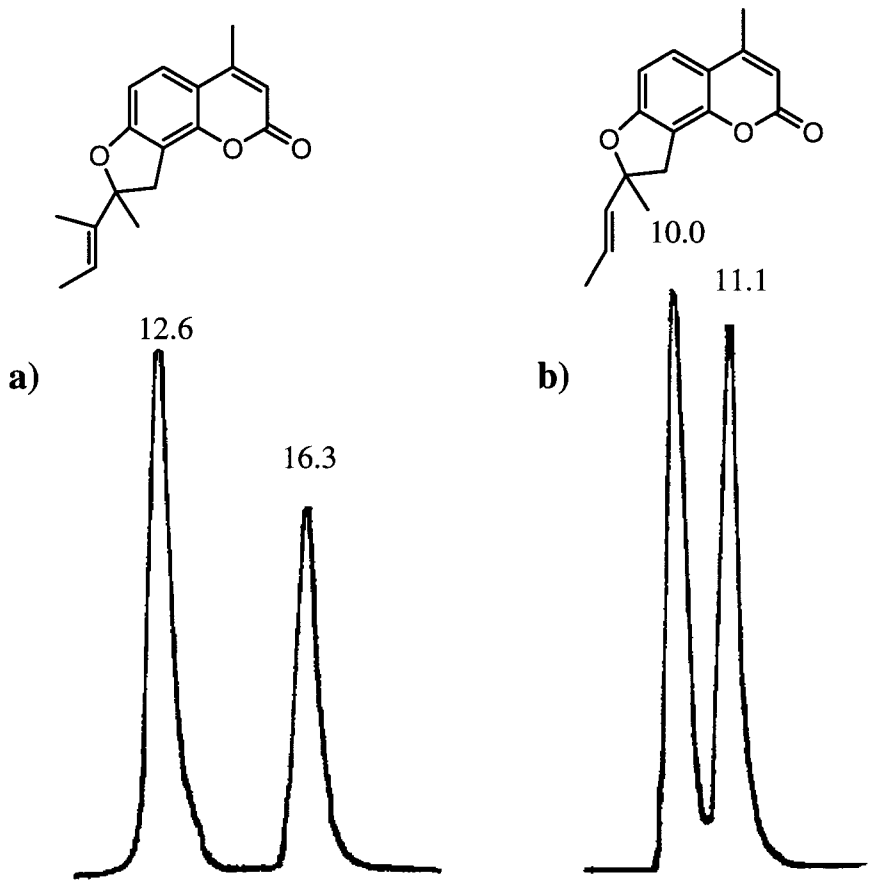



**Figure 5.** The effect of steric bulk on the enantioseparations. Separations performed on the Cyclobond RSP CSP with a 30/70 ACN/water mobile phase.
a) Dihydrofurocoumarin 10. b) Dihydrofurocoumarin 11.

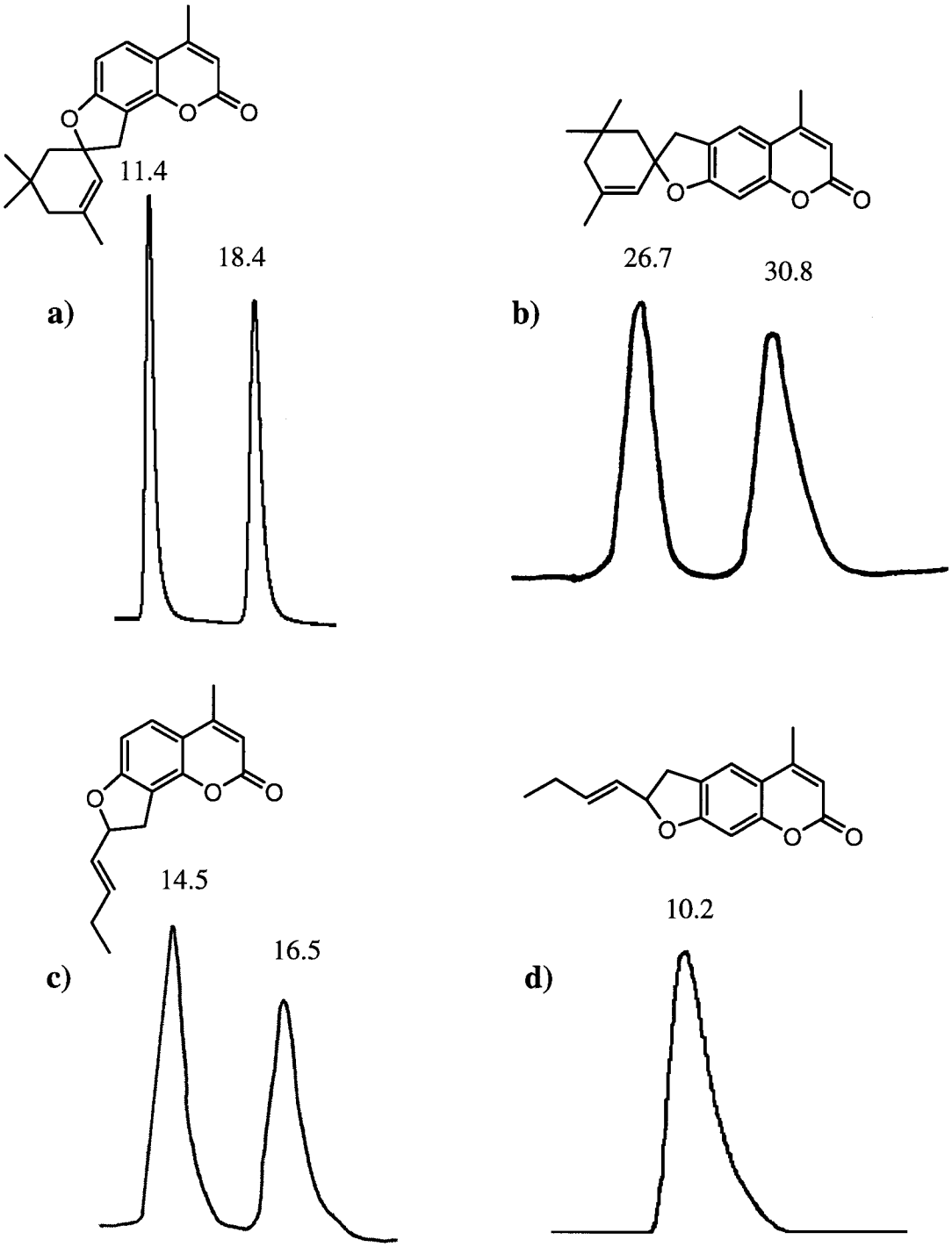



**Figure 6.** Angelicin/Psoralen analogue enantioseparations. Separations of dihydrofurocoumarins 13 and 25 performed on the Cyclobond RSP CSP. Separations of dihydrofurocoumarins 8 and 20 performed on the Cyclobond AC CSP. a) Angelicin analogue 13. b) Psoralen analogue 25. c) Angelicin analogue 8. d) Psoralen analogue 20.

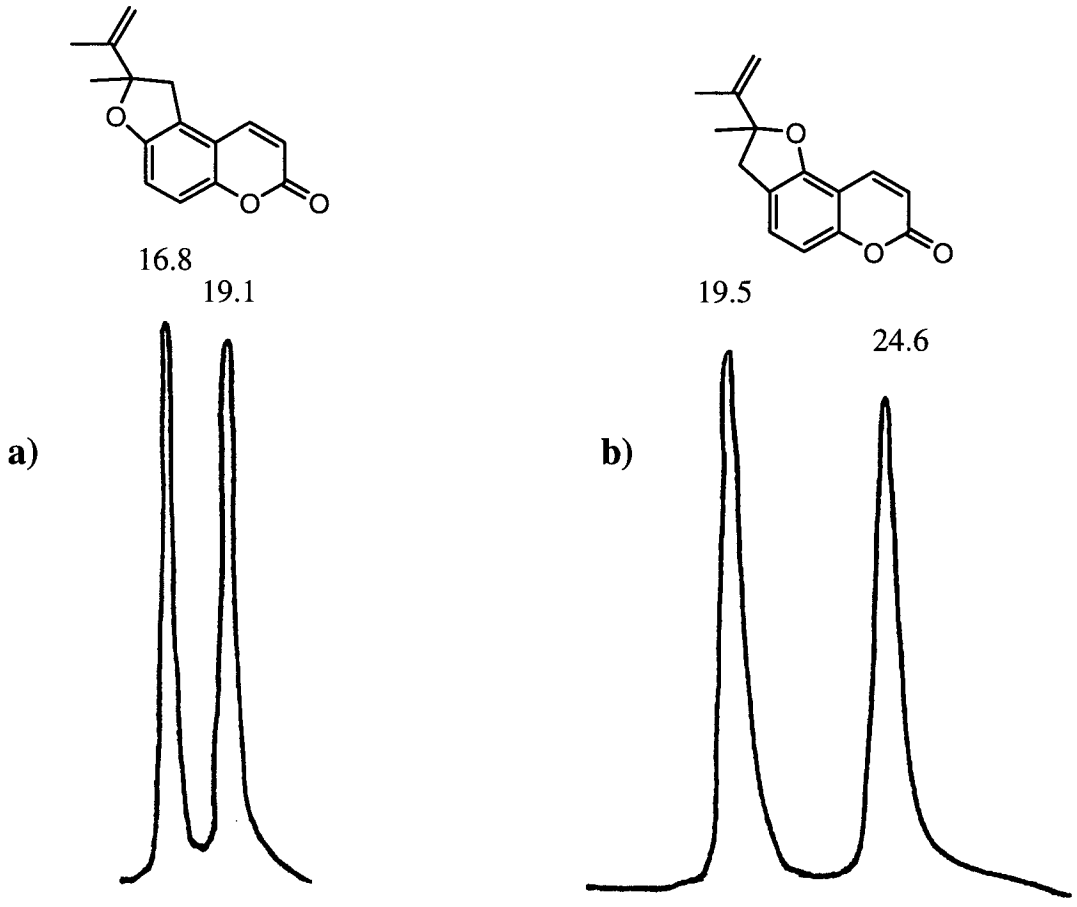
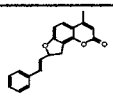



**Figure 7.** The effect of the dihydrofuran orientation on the separation of enantiomers on the Cyclobond RSP CSP. a) Dihydrofurocoumarin 17. b) Dihydrofurocoumarin 18.

**Table 1.** Retention factor (k'), enantioselectivity ($\alpha$) and enantioresolution ($R_s$) of all dihydrofurocoumarins on Cyclobond I 2000AC, DM, and RSP CSPs

| | | Cyclobond AC | | | | Cyclobond DM | | | | Cyclobond RSP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound # | Structure | k | a | Rs | Mobile Phase [a] | k | a | Rs | Mobile Phase [a] | k | a | Rs | Mobile Phase [a] |
| 1 |  | 3.32 | 1.18 | 1.93 | A | 5.54 | | | D | 3.83 | 1.21 | 1.31 | K |
| 2 |  | 3.11 | 1.10 | 0.77 | A | 6.23 | 1.14 | 1.92 | D | 6.49 | 1.21 | 1.58 | K |
| 3 |  | 5.28 | 1.24 | 2.24 | A | 4.78 | 1.39 | 4.02 | A | 12.46 | 1.21 | 1.80 | K |
| 4 |  | 5.02 | 1.07 | 0.55 | A | 4.20 | | | A | 6.57 | 1.13 | 1.76 | J |
| 5 |  | 9.65 | 1.18 | 1.73 | B | 3.29 | | | A | 23.07 | 1.13 | 1.03 | D |
| 6 |  | 2.24 | | | A | 5.03 | 1.04 | 0.27 | A | 11.99 | | | I |
| 7 [b] |  | 5.54 | | | C | 5.14 | 1.40 | 3.31 | D | 6.70 | 1.57 | 5.67 | A |
| 8 |  | 4.33 | 1.17 | 1.63 | B | 4.13 | | | A | 6.99 | 1.08 | 0.66 | I |
| 9 |  | 6.24 | 1.23 | 2.10 | C | 5.77 | | | A | 6.16 | 1.09 | 0.64 | K |
| 10 |  | 10.20 | 1.25 | 2.62 | D | 8.38 | 1.19 | 1.43 | D | 7.26 | 1.41 | 3.20 | E |
| 11 |  | 6.50 | 1.16 | 1.65 | D | 6.82 | 1.17 | 1.56 | D | 11.34 | 1.14 | 1.75 | A |
| 12 |  | 4.46 | | | A | 15.16 | 1.05 | 0.68 | A | 20.75 | 1.04 | 0.30 | A |
| 13 |  | 10.41 | 1.15 | 2.47 | A | 6.49 | 1.24 | 1.64 | A | 2.25 | 1.61 | 6.25 | F |

[a] Mobile phase composition A) 20/80 ACN/water B) 30/70 MeOH/water C) 35/65 MeOH/water D) 15/85 ACN/water E) 50/50 MeOH/water F) 50/50 ACN/water G) 30/70 ACN/water H) 55/45 MeOH/water I) 40/60 MeOH/water J) 25/75 ACN/water K) 45/55 MeOH/water

[b] Separation of diastereomers

**Table 1.** (continued)

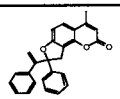
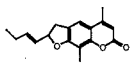
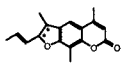
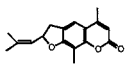
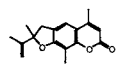
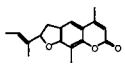
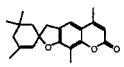
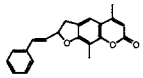
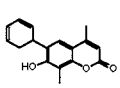
| | | Cyclobond AC | | | | Cyclobond DM | | | | Cyclobond RSP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound # | Structure | k | a | Rs | Mobile Phase [a] | k | a | Rs | Mobile Phase [a] | k | a | Rs | Mobile Phase [a] |
| 14 |  | 6.19 | 1.14 | 0.82 | I | 6.07 | | | J | 4.67 | 1.44 | 3.57 | G |
| 15 |  | 2.80 | 1.28 | 2.14 | B | 2.78 | 1.07 | 0.37 | C | 6.44 | 1.14 | 1.74 | C |
| 16 |  | 4.49 | 1.06 | 0.68 | G | 6.66 | 1.04 | 0.48 | D | 4.53 | 1.28 | 2.73 | J |
| 17 |  | 2.78 | 1.10 | 0.93 | G | 2.54 | 1.05 | 0.44 | D | 3.88 | 1.17 | 2.17 | I |
| 18 |  | 3.20 | 1.09 | 0.67 | G | 5.33 | 1.16 | 1.70 | D | 5.01 | 1.31 | 3.27 | A |
| 19 |  | 3.04 | 1.05 | 0.47 | I | 3.26 | | | A | 8.75 | 1.10 | 1.78 | A |
| 20 |  | 2.78 | | | A | 7.42 | | | D | 2.25 | | | I |
| 21 [b] |  | 2.44 | | | A | 5.81 | 1.12 | 1.37 | D | 1.82 | | | I |
| 22 |  | 2.75 | | | D | 8.77 | 1.07 | 0.74 | D | 17.40 | 1.08 | 1.26 | D |
| 23 |  | 4.61 | 1.07 | 0.51 | D | 6.35 | 1.24 | 2.42 | D | 19.38 | 1.11 | 1.69 | D |
| 24 |  | 2.75 | | | A | 8.49 | 1.06 | 0.76 | D | 4.74 | 1.07 | 0.85 | A |
| 25 |  | 6.80 | | | A | 10.25 | 1.14 | 1.16 | A | 9.63 | 1.24 | 2.08 | E |
| 26 |  | 1.74 | | | J | 2.79 | | | G | 19.21 | | | A |
| 27 |  | 2.12 | | | D | 4.51 | | | I | 5.03 | 1.18 | 1.70 | C |
| 28 |  | 2.09 | | | D | 1.62 | | | I | 2.34 | | | J |

**Table 2.** Retention factor (k'), enantioselectivity (a), and enantioresolution (Rs) for chiral dihydrofurocoumarins separated on Cyclobond RN, and DMP CSPs

| | Cyclobond DMP CD | | | |
|---|---|---|---|---|
| Compound # | k | α | Rs | Mobile Phase[a] |
| 10 | 8.70 | 1.11 | 1.34 | C |
| 11 | 4.45 | 1.05 | 0.33 | B |
| 19 | 3.84 | 1.04 | 0.76 | E |
| 20 | 7.11 | 1.04 | 0.32 | B |
| 26 | 4.71 | 1.32 | 2.38 | A |

| | Cyclobond RN CD | | | |
|---|---|---|---|---|
| Compound # | k | α | Rs | Mobile Phase[a] |
| 15 | 4.55 | 1.05 | 0.60 | D |
| 19 | 3.41 | 1.05 | 0.53 | E |

[a] Mobile Phase composition A) 75/25 MeOH/water C) 60/40 MeOH/water B) 55/45 MeOH/water D) 50/50 MeOH/water E) 40/60 ACN/water

# CYCLODEXTRIN-BASED LIQUID CHROMATOGRAPHIC ENANTIOMERIC SEPARATION OF CHIRAL DIHYDROFUROFLAVONES

A paper to be published in the Journal of Chromatography A

Douglas D. Schumacher[1, 2], Roman V. Rozhkov[3], Richard C. Larock[3],

and Daniel W. Armstrong[1]

## Abstract

A set of 13 racemic dihydrofuroflavone analytes in which the stereogenic center is located in the furan ring have been synthesized. Currently no effective asymmetric synthesis of this class of compounds exists. These compounds have various medicinal properties which are currently being investigated in several laboratories. The enantioselective separation of these dihydrofuroflavones by three native and six derivatized cyclodextrins has been evaluated in the reversed phase mode, the polar organic mode, and normal phase mode. Overall, 9 of the 13 dihydrofuroflavone analytes were baseline resolved (Rs > 1.5) on at least one of the cyclodextrin-based chiral stationary phases. The hydroxypropyl-β-cyclodextrin (Cyclobond I RSP) is the most effective chiral stationary phase (CSP) for the enantioseparations of these dihydrofurocoumarins analytes. It shows some enantioselectivity for 11 dihydrofuroflavones, and baseline separates 7 of the 13 compounds in the reversed phase mode. The 2,3-dimethyl-β-cyclodextrin also performed well, baseline separating 5 of the 13 analytes in the reversed phase mode.

---

[1] Graduate Student and Major Professor, respectively, Department of Chemistry Iowa State University.

[2] Primary researcher and author.

[3] Author in correspondence.

The native cyclodextrins showed no enantioselectivity for this class of compound in the reversed phase mode. The aromatic derivatized β-cyclodextrins are only marginally effective at separating the dihydrofuroflavone enantiomers in the reversed phase mode. The polar organic mode and the normal phase mode have also been evaluated with the native and aromatic derivatized β-cyclodextrins, but no enantioseparations were observed.

## Introduction

In the last decade flavones and flavonoids have been recognized by chemists, biologists, and pharmacologists as an emerging class of important compounds. Flavones and flavonoids are produced naturally by many plant species. Different substituted flavones have been found in red wine, onions, apples, teas, various berries [1-2], as well as in many tree barks and leaves [3-7].

Flavones and flavonoids have been shown to exhibit a vast array of beneficial physiological effects. The most significant of these are their antioxidant poroperties. Naturally occurring flavonoids such as quercetin, myricetin, and genistein can inhibit the oxidation of low density lipoproteins by scavenging free radicals [8,13], inhibit the oxidative damage of DNA [9], and stimulate DNA repair [11]. Other flavonoids have shown the ability to inhibit tumor growth [10] and inhibit blood platelet function [12]. Various human studies have shown that a diet rich in flavonoids is beneficial for the prevention and treatment of a wide variety of chronic diseases [14-17].

Recently, Rozhkov et al. have developed a synthesis for chiral dihydrofuroflavones via the palladium-catalyzed annulation of 1,3-dienes by *o*-iodoacetoxyflavonoids [18]. Substituents on the dihydrofuran portion of the heterocycle create a stereogenic center in the ring (Fig. 1c).

It is well know that the biological activity of enantiomeric compounds can vary greatly. Consequently, it has become standard practice to assess the disposition, function, and effect of each antipode of a chiral molecule [19]. As a consequence, most new products are produced and marketed as single isomers [19]. To date, there has been no investigation

of the biological activity of the individual enantiomers of chiral flavonoids. Also there is no reported, widely effective synthetic approach for these molecules. As such, methods must be developed to obtain both enantiomers in their pure form and to determine the activity of each.

Cyclodextrin based chiral stationary phases (CSPs) have been shown to be broadly applicable in their ability to separate enantiomers of a wide variety of compounds [20,21]. They are quite successful at resolving the enantiomers of chiral molecules with aromatic substituents or large aliphatic groups [22-26]. Furthermore, it has recently been shown that cyclodextrin-based CSPs are useful in the analysis of various chiral furocoumarin and dihydrofurocoumarin derivatives (which are a structurally related class of compounds) [27]. Consequently, cyclodextrin-based CSPs are a natural choice as CSPs for addressing the liquid chromatographic enantiomeric separation of these compounds.

## Experimental

### Materials

The CSPs (Cyclobond I, Cyclobond I AC, Cyclobond I DM, Cyclobond I DMP, Cyclobond I RN, Cyclobond I SN, Cyclobond I RSP, Cyclobond II, and Cyclobond III) were obtained from Advanced Separation Technologies (Whippany, NJ, USA). All stationary phases used consisted of the chiral selector bonded to 5 μm spherical silica gel. The chiral selectors used are underivitized cyclodextrins and the derivatized β-cyclodextrins, illustrated in Fig. 2. The dimensions of the columns are 250 × 4.6 mm. i.d. The triethylamine, methanol, acetonitrile, 2-propanol, and hexane were of HPLC grade and obtained from Fisher (Fairlawn, NJ, USA). Sodium chloride and acetic acid were ACS certified grade from Fisher. All substituted dihydrofuroflavones were prepared as previously reported [18].

## Equipment

The HPLC system used consisted of a quaternary pump, an auto sampler, a UV variable wavelength detector (1050, Hewlett Packard, Palo Alto, CA, USA), and an integrator (3395, Hewlett Packard). Mobile phases were degassed by ultra-sonication under vacuum for 10 minutes. UV detection was carried out at 220 nm. All separations were carried out at room temperature (~23°C).

## Column Evaluation

The performance of each stationary phase was evaluated and optimized in the reverse phase mode using acetonitrile/water and methanol/water mobile phases. The aromatic derivatized CSPs, Cyclobond DMP, RN, and SN, were also evaluated in the normal phase mode (isopropanol/hexane) and in the polar organic mode (100% acetonitrile). The composition of the mobile phase was optimized for each pair of enantiomers and at a flow rate of 1.0 mL $min^{-1}$.

## Calculations

Dead times ($t_M$) were estimated using the refractive index solvent peak on each CSP. Retention factors ($k$) were calculated using the equation $k = (t_r - t_M)/t_M$ where $t_r$ is the retention time of the analyte and $t_M$ is the dead time of the column. Enantioselectivity ($\alpha$) was calculated using the equation $\alpha = k_2/k_1$ where $k_1$ and $k_2$ are the retention factors of the first and second eluting enantiomers. Resolution factors ($R_s$) were calculated using the equation $R_s = 2 \times (t_r2 - t_r1) / (w_1 + w_2)$, where $t_r2$ and $t_r1$ are the retention times of the second and first enantiomers respectively and $w_1$ and $w_2$ are the base peak widths of the corresponding peaks.

## Results and Discussion

A series of 13 racemates were evaluated on nine different cyclodextrin based CSPs in the reversed phase mode (see Table I for structures and separation data). Figure 3 is a summary of the performance of each CSP in the reversed phase mode. Clearly the best CSP for these chiral dihydrofuroflavones utilized hydroxypropyl-β-cyclodextrin as the chiral selector (Cyclobond I RSP). The 2,3-dimethyl-β-cyclodextrin (Cyclobond I DM) based CSPs was also able to separate a large number of dihydrofuroflavones. The remaining CSPs, native cyclodextrins and aromatic derivatized β-cyclodextrin, were either ineffective or showed enantioselectivity only for a small number of the examined dihydrofuroflavone compounds in the reversed phase mode. A partial separation of enantiomers is reported in Fig. 3 if there is an observable enantioselectivity ($\alpha > 1.02$) and a baseline separation of enantiomers is reported if the peak-to-peak resolution ($R_s$) exceeds 1.5.

The effect of mobile phase composition was also investigated. All 13 compounds were analyzed in the reversed phase mode with both acetonitrile/water and methanol/water mobile phases on all CSPs. Generally, comparable results for enantioselectivity and resolution were obtained with each solvent system; however, there were several cases where an acetonitrile/water mobile phase successfully separated enantiomers where the methanol/water mixture failed. This is thought to be due to hydrogen bonding of the methanol molecules to the hydroxyl groups on the cyclodextrin, which may interfere with the enantioselective association process. The opposite effect was observed for the aromatic derivatized cyclodextrin CSPs as methanol/water mobile phases were more useful at separating the enantiomers of chiral dihydrofuroflavones than the acetonitrile/water mobile phases (Table II). The effect of pH (4.00, 5.00, 6.00, 7.00, and unbuffered [pH = 6.20], 0.1% (*v/v*) triethylamine/acetic acid) and ionic strength (0 M, 0.10 M, 0.20, 0.30, 0.40, and 0.50 M NaCl) were also investigated. However, these type of mobile phase modifications did not affect the selectivity or resolution (data not shown). This result is likely due to the fact that the dihydrofuroflavones are neutral, hydrophobic compounds with no ionizable groups (see Fig. 1 and Table I).

**Cyclobond I RSP and DM Chiral Stationary Phases**

Table I summarizes the separation data for the Cyclobond I DM and Cyclobond I RSP columns in the reversed phase mode of operation. The structure of each dihydrofuroflavone and the optimal mobile phase compositions are given, as well as the values for *k*, *Rs*, and $\alpha$.

It is well known that cyclodextrin CSPs excel at enantioseparations where analytes have bulky groups near the stereogenic center [22-26]. For example, the separation of analytes 1, 2, and 3 clearly shows that changing the local environment near the stereogenic center effects the separation on both of the non-aromatic derivatized β-cyclodextrin CSPs (Table I). The resolution of these compounds on the hydroxypropyl-β-cyclodextrin CSP is decreased with the addition/removal of a single methyl- group (Fig. 4). This is supported by the separations of compounds 1 and 2. It is also of interest to note that the removal of a methyl group beta to the chiral center is detrimental to the observed separation (compounds 2 and 3).

The trends on the 2,3-dimethyl-β-cyclodextrin (Cyclobond I DM) are quite different from those on the hydroxypropyl β-cyclodextrin (Cyclobond I RSP). For example, a comparison of the separations of compounds 1 and 2 show that the addition of a methyl group on the chiral center greatly enhances chiral recognition and enantioresolution. It is also seen that the removal of a methyl- group beta to the stereogenic center is slightly detrimental to the observed separation as shown by the data from compounds 2 and 3 (Fig. 5).

In all cases, these methyl groups create an additional steric interaction near the stereogenic center which can greatly affect chiral recognition. Other examples of this interaction are shown in the separation of compounds 8 and 11. These compounds have a large amount of steric bulk near the chiral centers which is known to enhance chiral recognition. These same groups are also quite rigid which eliminates the rotational movement of the groups about the stereogenic center which is also beneficial to chiral recognition [21]. Conversely, compounds 5 and 10 have little steric bulk near the chiral center and a high degree of rotational mobility leading to diminished chiral selectivity and

enantioresolution. Therefore, steric interactions and molecular rigidity must play a significant role in the selectivity of this class of compounds.

The enantioseparations of the structural isomers of the dihydrofuroflavones are also of interest. While these analytes are quite similar, the more linear dihydrofuroflavone derivatives in which the furan moiety is attached at the 6,7 position are generally much less well resolved than their more angular structural isomers (attached at the 8,9 position). This is shown in Fig. 6 for compounds 11 and 12. The more angular dihydrofuroflavone (compounds 11) is more easily separated into its enantiomers on each stationary phase. This same trend is shown when comparing the separation of compounds 4 and 13. The difference in the observed enantioselectivity between these groups of compounds must be due to the spatial orientation of the dihydrofuran group. This limits the orientational or reorientational ability of the analyte in the inclusion complex as well as giving another point of steric interaction between the molecule and the substituents on the mouth of the cyclodextrin cavity.

**Other CSPs – Native and Aromatic Derivatized Cyclodextrins**

Other cyclodextrin-based CSPs were less useful in separating enantiomers of these types of compounds in the reversed phase mode. The remaining CSPs can be divided into aromatic derivatized cyclodextrin CSPs (Cyclobond I RN, SN and DMP) and native cyclodextrin CSPs (Cyclobond I, II, and III). The results of these analyses are presented in Table II. The aromatic derivatized cyclodextrins gave the best separation of any of the analytes investigated (compound 10). This is due to the phenyl- substituent on the stereogenic center competing as an alternate site for inclusion complexation. As other analyses with the aromatic derivatized cyclodextrins were less successful, it is reasonable to conclude that an excess of aromatic steric bulk on the chiral selector is detrimental to the enantioseparation of most chiral dihydrofuroflavones. The native cyclodextrins did not show any selectivity for any of the analytes investigated.

**Normal Phase and Polar Organic Modes**

The normal phase mode was investigated on all aromatically derivatized CSPs. The Cyclobond I RN, SN, and DMP columns were each evaluated with a 5/95 isopropanol/hexane mobile phase. All analytes were appreciably retained, but no enantioselectivity was observed. The polar organic mode was also investigated under the weakest condition (100% acetonitrile) where all compounds eluted at the dead time of the column.

**Mechanistic Observations**

The binding of a dihydrofuroflavone molecule to a cyclodextrin CSP is a dynamic process. Both the furan portion and the flavone portion of a dihydrofuroflavone molecule compete to enter the cyclodextrin cavity and form an inclusion complex in the reverse phase mode. However, only one of these two inclusion complex orientations will produce the enantioselectivity which leads to an enantiomeric separation. It is well established that, for a cyclodextrin to form an enantioselective diastereomeric complex, the substituents off of the stereogenic center of the analyte must be in close proximity to the secondary hydroxyls at the mouth of the cyclodextrin (in the case of the native cyclodextrins) or be able to interact with the pendant groups on the derivatized cyclodextrin in order to achieve the necessary three-points of interaction [21,26,28]. If the furan portion of the molecule complexes within the cavity of the cyclodextrin, the stereogenic center will be buried inside the cyclodextrin torus. This arrangement will not be conducive to chiral recognition as the substituents on the chiral center will be unable to interact with these hydroxyls (or derivative groups) on the mouth of the cyclodextrin cavity. Therefore, for chiral recognition to occur, the flavone portion of the analyte molecule must occupy the cyclodextrin cavity thereby allowing the furan portion which contains the stereogenic center to be in close proximity to the mouth of the cyclodextrin cavity where it can interact with the secondary hydroxyls or derivative arms on the torus of the cavity.

The size of these analytes (Table I and II) supports the contention that the same portion of these polycyclic analytes must protrude above the cyclodextrin cavity when an inclusion complex is formed. The hydroxypropyl-β-cyclodextrin CSP (Cyclobond I RSP) is very successful at resolving larger analytes where significant portions of the included molecule protrude from the mouth of the cyclodextrin [25, 29], whereas native cyclodextrins are not. The hydroxypropyl groups of the derivatized cyclodextrins are also known to extend well beyond the mouth of the cyclodextrin cavity [28] and are in a position to interact with both the dihydrofuran moiety and any substituents attached to the stereogenic center via steric interactions or hydrogen bonding. These interactions have previously been shown to be the most prominent interactions that lead to enantioselectivity in the cases where the hydroxypropyl-β-cyclodextrin CSP is superior to the native β-cyclodextrin CSP [29]. Therefore, the additional interactions produced by these derivative groups are essential for chiral recognition. Taking into consideration the fact that native cyclodextrins CSPs are completely ineffective in separating these compounds, one must conclude that when the dihydrofuroflavones form an enantioselective inclusion complex with a derivatized cyclodextrin in the reversed phase mode, the stereogenic center must be in close proximity to the mouth of the cyclodextrin selector.

## Conclusions

The Cyclobond DM and RSP are the most effective cyclodextrin-based CSPs for separating the enantiomers of chiral dihydrofuroflavones. This selectivity arises from the ability of the substituent groups at the mouth of the cyclodextrin cavity to interact with the substituent groups attached to the stereogenic center of analyte molecule. The effect of steric bulk near the chiral center and the rigidity of the molecule both have a pronounced effect on enantioselectivity. The spatial placement of the furan moiety about the parent flavone molecule also has a significant impact on the separation achieved. Generally, the more angular type flavones are better resolved into enantiomers than the more linear structural isomers. The aromatic derivatized and native cyclodextrins produced the best separation of any analyte in this study, but no other baseline enantioseparations were seen on these CSPs. The normal phase and polar organic modes of operation did not resolve any of these compounds into enantiomers with the CSPs investigated.

## Acknowledgements

Support of this work by the National Institutes of Health, NIH RO1 GM53825-06 is gratefully acknowledged.

R. V. Rozhkov and R. C. Larock also acknowledge the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research and Johnson Matthey Inc. and Kawaken Fine Chemicals Co., for donations of palladium acetate.

## References

[1] M.G.L. Hertog, P.C Hollman, M.B. Katan, J. Agric Food Chem. 40 (1992) 2379-83.

[2] M.G.L. Hertog, P.C. Hollman, P. van de Putte, J. Agric Food Chem. 41 (1993) 1242-6.

[3] B.T. Ngadjui, E. Dongo, H. Tamboue, K. Fogue, B.M. Abegaz, Phytochem. 50 (1999) 1401-1406.

[4] B.T. Ngadjui, S.F. Kouam, E. Dongo, G.W.F. Kapche, B.M Abegaz, Phytochem. 55 (2000) 915-919.

[5] Wen-Kui Li, Pei-Gen Xiao, Ru-Yi Zhang, Phytochem. 38 (1995) 807-808.

[6] P. Maximo, A. Lourenco, S. Savluchinske Fieo, J.C. Roserio, J. Nat. Prod. 65 (2002) 175-178.

[7] D. Lee, K.P.L Bhat, H.H.S. Fong, N.R. Farnsworth, J.M. Pezzuto, A.D. Kinghorn, J. Nat. Prod. 64 (2001) 1286-1293).

[8] C. de-Whalley, S.M. Rankin, J.R.S Hoult, W. Jessup, D.S. Leake, Biochem. Pharmacol, 39 (1990) 1743-1750.

[9] M.K. Johnson, G. Loo, Mutat. Res. 459 (2000) 211-218.

[10] R.L. Schleicher, C.A. Lamartiniere, M. Zheng, M. Zhang, Cancer Lett. 136 (1999) 195-201.

[11] V. Abalea, J. Cillard, M.P. Dubois, O. Sergent, P. Cillard, I. Morel, Free Radic. Biol. Med. 26 (1999) 1457-1466.

[12] P. Pignatelli, F.M. Pulcinelli, A. Celestini, L. Lenti, A. Ghiselli, P. P. Gazzaniga, F. Violi, Am. J. Clin. Nutr. 72 (2000) 1150-1155.

[13] E. Frankel, J. Kanner, J. German, E. Parks, J. Kinsella, Lancet. 341 (1993) 454-457.

[14] G.T. McAnlis, J. Mc.Eneny, J. Pearce, I. Young, Eur. J. Clin. Nutr. 53 (1995) 92-96.

[15] B.J. Boersma, S. Barnes, M. Kirk, C.C. Wang, M. Smith, H. Kim, J. Xu, R. Patel, V.M. Darley-Usmar, Mutat. Res. 480-481 (2001) 121-127.

[16] J.D. O'Reilly, A.I. Mallet, G.T. McAnlis, I.S. Young, B. Halliwell, T.A.B. Sanders, H Wiseman, Am. J. Clin. Nutr. 73 (2001) 1040-1044.

[17] M. Chopra, P. Fitzsimons, J.J. Strain, D.I. Thurnham, A.N. Howard, Clin Chem. 46 (2000) 1162-70.

[18] R. V. Rozhkov, R. C. Larock, Org. Lett. (2003) In Press.

[19] United States Food and Drug Administration, Chirality 4 (1992) 338-340.

[20] D. W. Armstrong, W. DeMond, J. Chromatogr. Sci. 22 (1984) 411-415.

[21] D. W. Armstrong, T. J. Ward, R. D. Armstrong, T. E. Beesley, Science 232 (1986) 1132-1135.

[22] C. R. Mitchell, M. Desai, R. McCulla, W. Jenks, D. W. Armstrong, Chromatographia 56 (2002) 127-135.

[23] D. W. Armstrong, J. Zukowski, J. Chromatogr. A 666 (1994) 445-448.

[24] D. W. Armstrong, T. J. Ward, A. Czech, B. P. Czech, R. A. Bartsch, J. Org. Chem. 50 (1985) 5556-5559.

[25] D. W. Armstrong, L. W. Chang, S. C. Chang, X. Wang, H. Ibrahim, G. R. Reid, T. E. Beesley, J. Liq. Chromatogr. Related Technol. 20 (1997) 3279-3295.

[26] D. W. Armstrong, W. DeMond, B. P. Czech, Anal. Chem. 57 (1985) 481-484.

[27] D.D. Schumacher, C.R. Mitchell, T.L Xiao, R.V. Rozhkov, R.C. Larock, D.W. Armstrong, J. Chromatogr. A. 1011 (2003) 37-47.

[28] W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak, T. J. Ward, Anal. Chem. 57 (1985) 237-242.

[29] A. M. Stalcup, S. C. Chang, D. W. Armstrong, J. Pitha, J. Chromatogr. 513 (1990) 181-194.

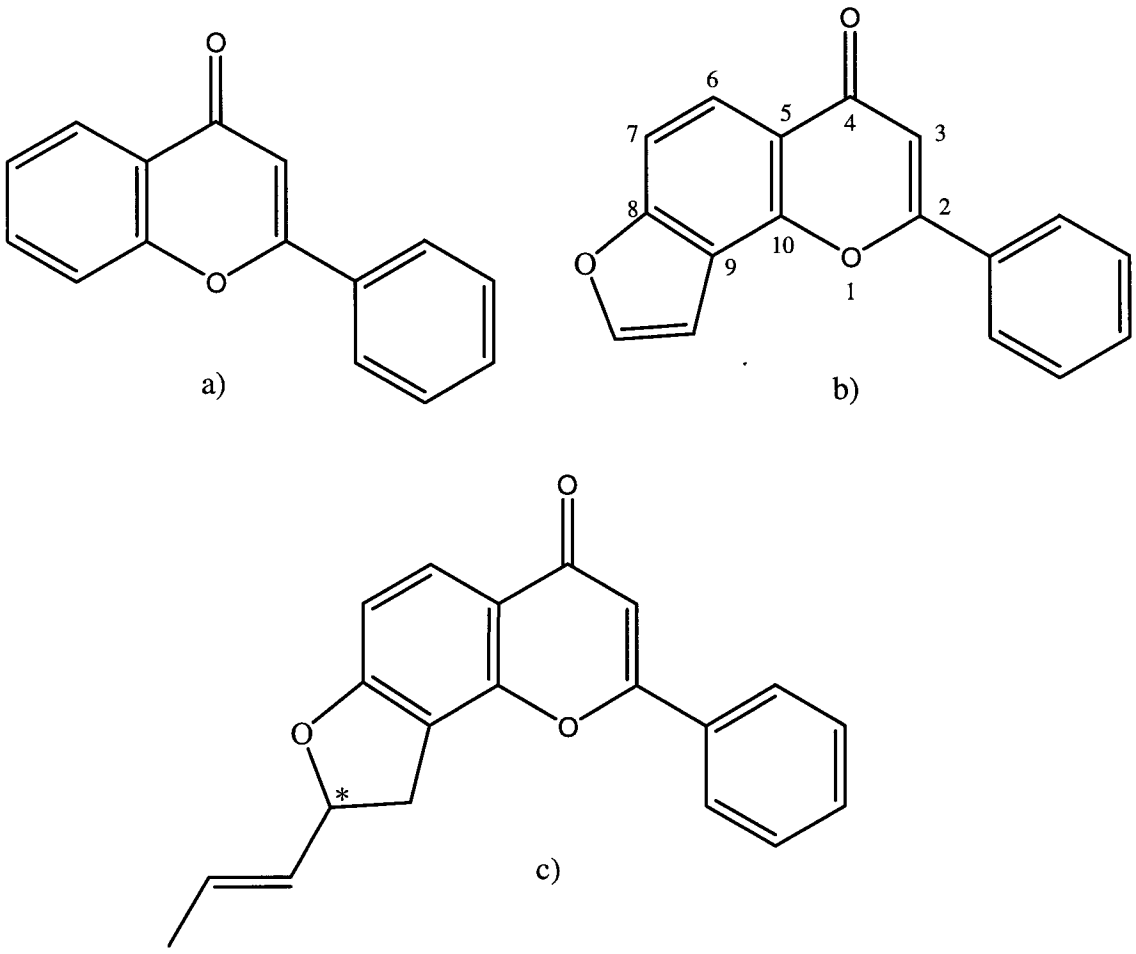



**Figure 1.** a) Flavone structure. b) Dihydrofuroflavones type compounds are derivatives of flavones where the furan ring is fused to the 7,8-segment of the flavone. The structure is numbered as the parent flavone for consistency of comparisons in the discussion. c) A chiral, substituted dihydrofuroflavone analogue where the stereogenic center is denoted by an asterisk.

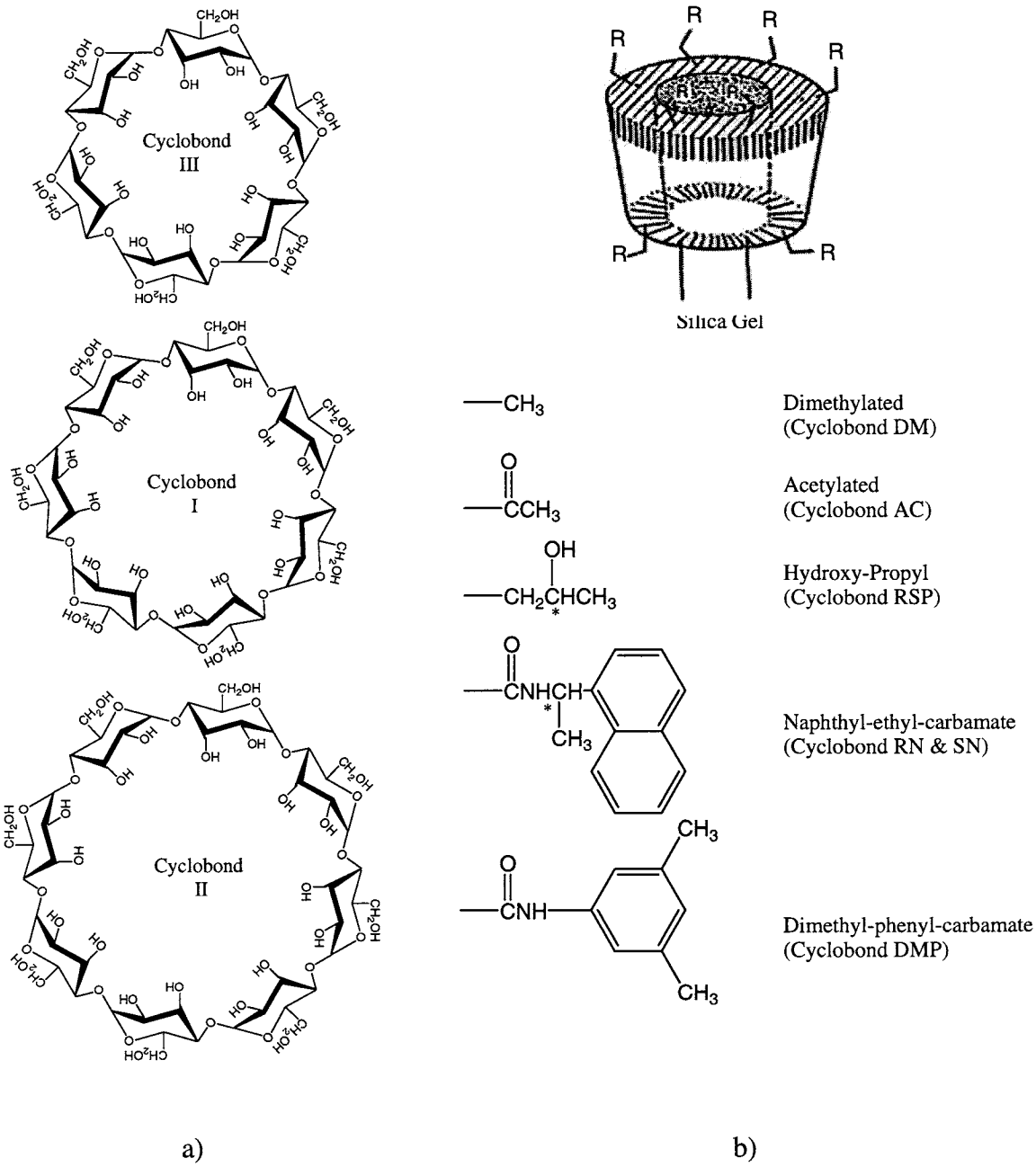



**Figure 2.** a) Native alpha, beta, and gamma cyclodextrins (i.e. Cyclobond III, I and II respectively). b) Types of derivatized cyclodextrins. An asterisk denotes the stereogenic center. Reproduced from Reference 27.

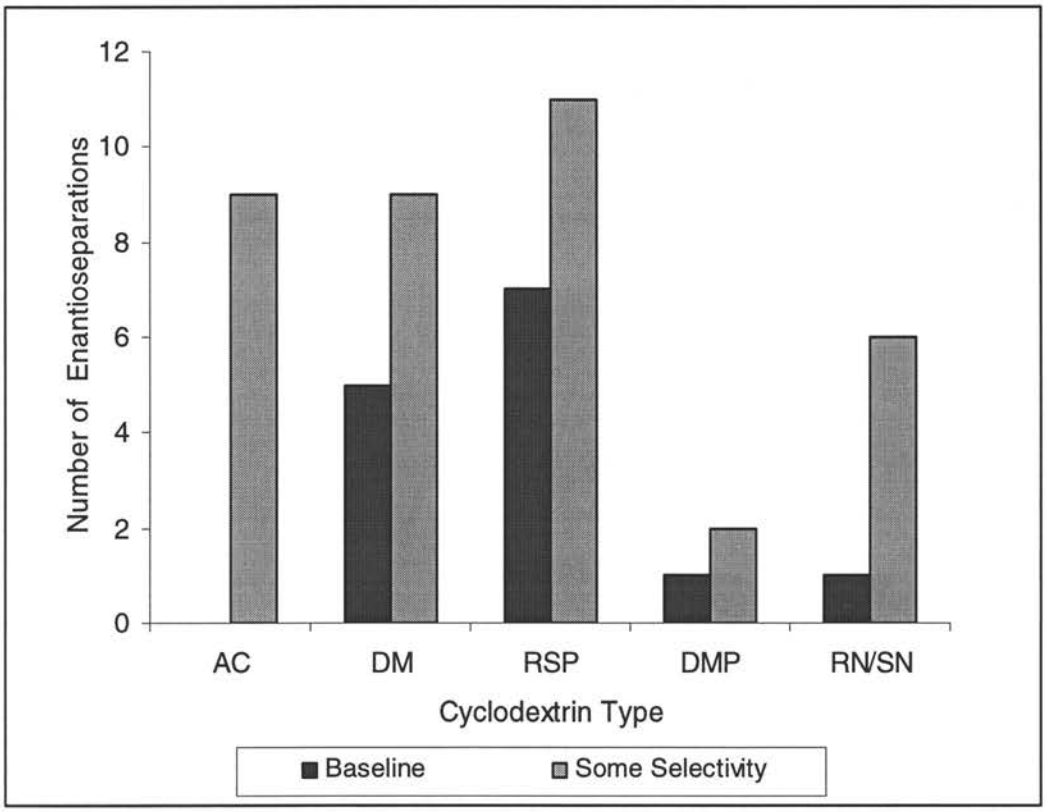



**Figure 3.** Number of separations in the reversed phase mode using cyclodextrin-based CSPs. The various types of cyclodextrins and their designated abbreviations are illustrated in Figure 1. Grey Bars: number of observable enantioselective separations, enantioselectivity, $\alpha \geq 1.02$. Black Bars: number of baseline separations, enantioresolution, $Rs \geq 1.5$.

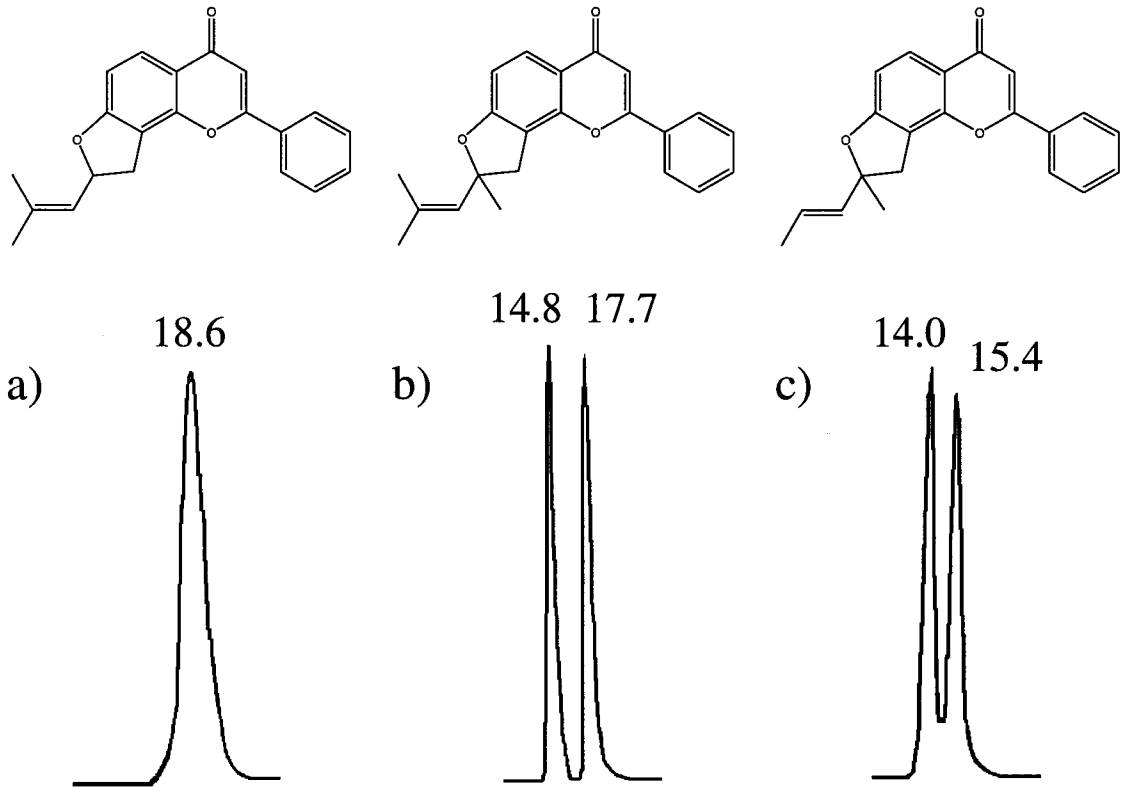



**Figure 4.** The effect of steric interactions. Separations performed on the Cyclobond RSP CSP with a 45/55 MeOH/water mobile phase. a) Dihydrofuroflavone 1. b) Dihydrofuroflavone 2. c) Dihydrofuroflavone 3.

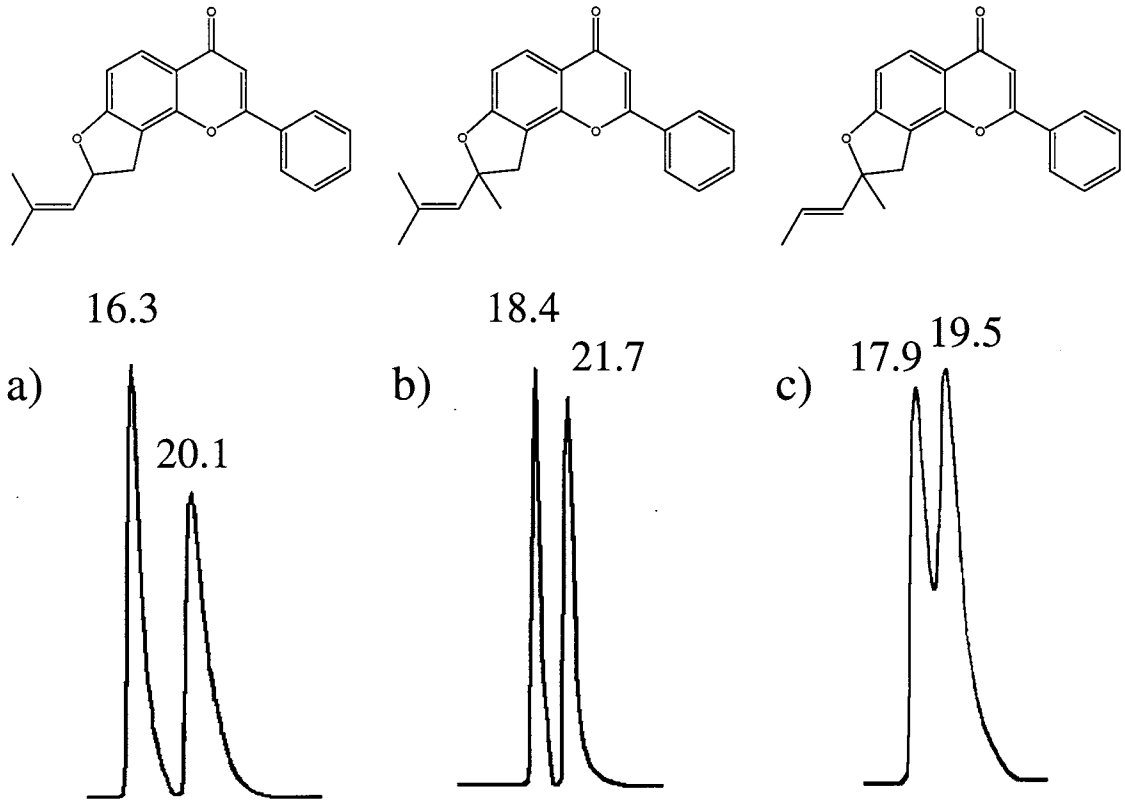



**Figure 5.** The effect of steric interactions. Separations performed on the Cyclobond DM CSP with a 35/65 MeOH/water mobile phase. a) Dihydrofuroflavone 1. b) Dihydrofuroflavone 2. c) Dihydrofuroflavone 3.

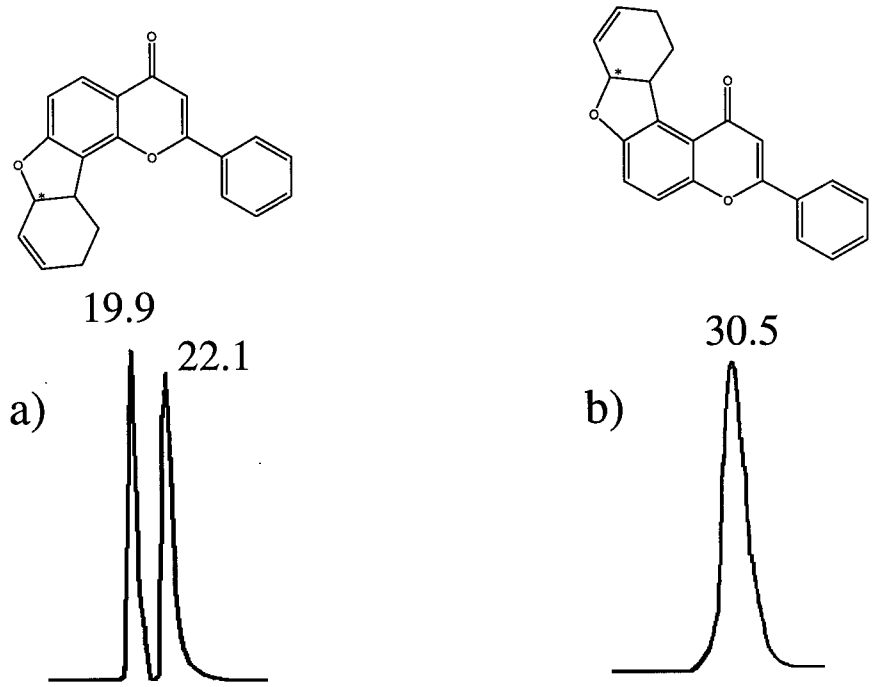
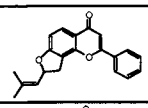
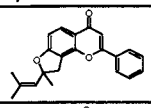
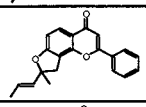
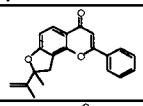
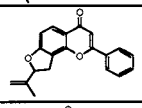
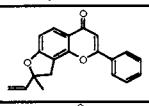
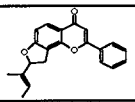
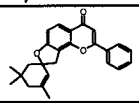
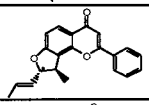
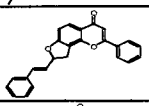
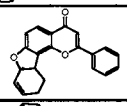
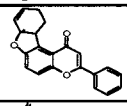
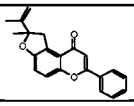



**Figure 6.** The effect of furan orientation. Separations performed on the Cyclobond RSP CSP with 15/85 ACN/water mobile phase in case a and 20/80 ACN/water mobile phase in case b. a) Dihydrofuroflavone 11. b) Dihydrofuroflavone 12.

**Table 1.** Retention factor (k'), enantioselectivity (α) and enantioresolution ($R_s$) of all dihydrofurocoumarins on Cyclobond I 2000 DM and RSP CSPs,

| | | Cyclobond DM | | | | | Cyclobond RSP | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Compound # | Structure | k | α | Rs | Mobile Phase [a] | | k | α | Rs | Mobile Phase [a] |
| 1 |  | 3.79 | 1.30 | 1.78 | E | | 3.10 | | | E |
| 2 |  | 3.10 | 1.19 | 1.69 | B | | 3.36 | 1.25 | 2.15 | D |
| 3 |  | 2.62 | 1.11 | 1.00 | B | | 2.03 | 1.21 | 1.99 | B |
| 4 |  | 2.46 | 1.08 | 0.67 | B | | 7.03 | 1.06 | 0.71 | B |
| 5 |  | 2.33 | | | B | | 2.34 | 1.05 | 1.02 | A |
| 6 |  | 2.61 | | | B | | 6.40 | 1.15 | 1.83 | B |
| 7 |  | 3.40 | 1.11 | 0.73 | E | | 3.83 | 1.20 | 2.21 | A |
| 8 |  | 2.76 | 1.21 | 1.50 | A | | 2.12 | 1.31 | 2.25 | F |
| 9 |  | 3.21 | 1.63 | 3.44 | B | | 3.26 | 1.66 | 4.00 | D |
| 10 |  | 5.59 | 1.10 | 1.03 | D | | 7.11 | | | A |
| 11 |  | 4.62 | 1.19 | 1.64 | C | | 4.86 | 1.13 | 1.59 | B |
| 12 |  | 3.26 | | | B | | 12.73 | 1.14 | 1.29 | F |
| 13 |  | 3.13 | | | B | | 7.84 | 1.05 | 0.71 | A |

[a] Mobile phase composition A) 25/75 ACN/water B) 20/80 ACN/water C) 15/85 ACN/water D) 45/55 MeOH/water E) 40/60 MeOH/water F) 35/65 MeOH/water

**Table 2.** Retention factor (k'), enantioselectivity (a), and enantioresolution (Rs) for chiral dihydrofurocoumarins separated on Cyclobond RN, SN, and DMP CSPs

| | Cyclobond DMP CD | | | |
|---|---|---|---|---|
| Compound # | k | α | Rs | Mobile Phase[a] |
| 4 | 12.19 | 1.09 | 1.20 | D |
| 10 | 6.21 | 1.37 | 4.27 | A |

| | Cyclobond RN CD | | | |
|---|---|---|---|---|
| Compound # | k | α | Rs | Mobile Phase[a] |
| 6 | 5.72 | 1.03 | 0.58 | D |
| 7 | 4.22 | 1.02 | 0.25 | F |
| 9 | 3.99 | 1.02 | 0.30 | F |
| 10 | 6.78 | 1.12 | 1.82 | B |
| 11 | 1.87 | 1.03 | 0.53 | B |
| 12 | 4.50 | 1.02 | 0.41 | F |

| | Cyclobond SN CD | | | |
|---|---|---|---|---|
| Compound # | k | α | Rs | Mobile Phase[a] |
| 4 | 9.62 | 1.06 | 1.11 | E |
| 9 | 9.51 | 1.07 | 1.38 | E |
| 10 | 10.91 | 1.46 | 5.13 | C |
| 11 | 7.43 | 1.06 | 1.11 | E |
| 12 | 9.64 | 1.02 | 0.44 | G |

[a] Mobile Phase composition A) 75/25 MeOH/water B) 70/30 MeOH/water C) 65/35 MeOH/water, D) 60/40 MeOH/water E) 50/50 MeOH/water F) 45/55 MeOH/water G) 35/65 ACN/water

# CONCLUSIONS

Cyclodextrin based CSPs are useful for the enantioseparation of chiral dihydrofurocoumarins and chiral dihydrofuroflavones. The most effective cyclodextrin based CSPs for these separations in the reversed phase mode are the acetyl-β-cyclodextrin (Cyclobond I AC), the 2,3-dimethyl-β-cyclodextrin (Cyclobond I DM), and the hydroxypropyl-β-cyclodextrin (Cyclobond I RSP).

The observed selectivity is mainly due to the orientation of the analyte-cyclodextrin complex. The analyte molecules must complex with these chiral selectors in such a way that the substituents off of the center located in the furan ring can interact with the derivative moieties on the cyclodextrin molecule. This conjecture is supported by the fact that the native cyclodextrin CSPs were unable to separate any of the compounds examined. Additionally, these types of molecules can bind into the cyclodextrin cavity at either the furan end or the lactone end of the. Both of these orientations contribute to the retention of the analyte, however, only the complexation of the lactone end of the molecule into the cyclodextrin cavity will give rise to the correct orientation for the substituents on the chiral center and the derivative arms on the cyclodextrin to interact. In a small number of cases, the linear derivatives of the dihydrofuroflavones and dihydrofurocoumarins (psoralens) are also resolved to a diminished extent. This result is again due to the interaction between the analyte molecules and the derivative moieties on the cyclodextrin, however, these types of molecules are more conformationally mobile inside of the cyclodextrin cavity. This contributes to fewer of this type of analyte being baseline resolved on these CSPs.

Several other interactions also have a pronounced effect on the observed selectivity. The presence of steric bulk and the rigidity of the substituents about the chiral center have been shown to greatly enhance the chiral recognition of these analytes. Additionally, the orientation of the furan oxygen and the spatial placement of the dihydrofuran moiety about the parent coumarin or flavone molecule both play a significant role in the selectivity of the separation.

All of these factors taken together support the accepted mechanism of chiral recognition on cyclodextrin based CSPs. In the reversed phase mode of operation, more angular dihydrofurocoumarins (angelicins) and dihydrofuroflavones were better resolved into enantiomers than their more linear counterparts. This supports the contention that these molecules must include into the cyclodextrin cavity in an orientation such that the substituents on the chiral center can interact with the hydroxyl groups or derivative arms on the mouth of the cyclodextrin cavity [1]. Since the native β-cyclodextrin did not resolve the enantiomers of any of the compounds investigated; one must logically conclude that the interaction with these derivative groups is necessary for the chiral recognition for these analytes.

No separations for either dihydrofurocoumarins or dihydrofuroflavones were seen in the polar organic mode of operation. Since neither type of molecule has good hydrogen bonding groups near the chiral center, it makes good sense that these molecules would not be resolved in this mode [2].

Finally, no separations were seen for any of the molecules investigated in the normal phase mode of operation. Both of these classes of molecule contain an aromatic group which is a necessary structural feature for molecules to be separated in this mode by these CSPs, however, it is possible that this interaction does not give rise to the required three points of interaction about the chiral center [3].

In closing, cyclodextrin based CSPs are quite applicable to the enantioseparation of racemic mixtures of chiral dihydrofurocoumarins and chiral dihydrofuroflavones. This method can be used for a preparative separation of the enantiomers of these compounds, which will allow for the assessment of their individual biological activities. If any of the enantiomers of these compounds show promising medicinal effects, this analytical approach could be used for the development of new drugs for the treatment of various diseases.

## References

[1] A. M. Stalcup, S. C. Chang, D. W. Armstrong, J. Pitha, Journal of Chromatography, 513 (1990) 181-194.

[2] S. C. Change, G. L. Reid, S. Chen, C. C. Chang, D. W. Armstrong, Trends in Analytical Chemistry, 12 (1993) 144-153.

[3] D. W. Armstrong, A. M. Stalcup, M. L. Hilton, J. D. Duncan, J. R. Faulkner, S. C. Chang, Analytical Chemistry, 62 (1990) 1610-1615.

## ACKNOWLEDGEMENTS

I would like to thank Dr. Daniel W. Armstrong, Cliff Mitchell, Grant Edwards, Kale and Steph Swainston, Jared Anderson, Mike Rodriguez, Roman Rozhkov, Tom Xiao, Bo Zhang, Chris and Christine Aikens, Jack Barrow, and Rachel Millen for all of the trials and tribulations in the last 3 years. I would also like to thank Pepsi Cola Int. for Mountain Dew that kept me awake while I was working. Thanks to Da Vinci's, May House, and Hickory Park for good food and delivery. Lastly, I want to thank my soon to be (December 6, 2003) wife Emily for putting up with all of the ups and downs in grad school as well as for the times she made me "baked goods" ☺.