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Review

Chiral derivatizing reagents for drug enantiomers bearing hydroxyl groups

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Abstract

This review extensively summarizes and critically evaluates the recent research on the indirect resolution technique for enantiomeric alcohols. Twenty-one chiral derivatizing reagents divided in seven types, including chiral acids, activated acids, chloroformates, isocyanates, carbonyl nitriles, oxazolidin-2-ones and lactones, are described. The derivatization methods of the various chiral reagents, the liquid chromatography separation systems, the detection systems used for the diastereomeric derivatives of alcohols as well as their limitations and the prospects of the indirect resolution technique for the future are thoroughly discussed. This paper aims to instruct the application of a particular chiral reagent and the technical approach to be used and should be beneficial to the development of an indirect resolution method for enantiomeric alcohols as well as to the biomedical investigation of the differences between the antipodes of chiral alcohols.

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List of abbreviations

BNCN	(−)- or (+)-2-Methyl-1,1′-binaphthalene-2′-carbonyl nitrile
CAC	(−)-Camphanic acid
CBP	Carbobenzyloxy-L-proline
CDR	Chiral derivatizing reagent
CMP	Chiral mobile phase
CSC	Camphorsulfonyl chloride
CSP	Chiral stationary phase
DBD	di-*n*-Butyltin diaurate
DBI	Dehydroabiethyl isocyanate
DBTAA	(*R*,*R*)-O,O-Dibenzoyltartaric acid anhydride
DCC	Dicyclohexyl carbodimide
DEA	N,N-Diethylamine
DMAP	N,N-(Dimethylamino) pyridine
DMEA	N,N-Dimethylethanolamine
DMF	N,N-Dimethylaminoformamide
EDPC	1-Ethyl-3[3-(dimethylamino)prolyl] carbodimide
HETE	Hydroxyeicosatetraenoic acid
HTMB	[3a*S*-(3aα,4a,7a,7aα)]-3a,4,5,6,7,7a-Hexahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2(3H)-on
MAC	Menthoxyacetyl chloride
MBI	α-Methoxybenzyl isocyanate
MCF	Menthyl chloroformate
MNC	(+)-6-Methoxy-α-methyl-2-naphthaleneacetyl chloride
MTPA-Cl	*R*-(+)-1-α-methoxy-α-trifluoromethylphenylacetyl chloride
NEIC	*S*- or *R*-1-(1-Naphthyl)ethyl isocyanate
NP	Normal-phase
NPC	D-2-(2-Naphthyl)-propionyl chloride
NSP-Cl	*S*(−)-N-1-(2-Naphthylsulfonyl)-2-pyrrolidinecarbonyl chloride
NSPI	1-N-(Naphthylsulfonyl)prolyl isocyanate
PEIC	*S*- or *R*-Phenylethyl isocyanate
RP	Reversed-phase
r.t.	Room temperature
POP	Pyrrolidinopyridine
TCA	Trichloroacetic acid
TEA	Triethylamine
THF	Tetrahydrofuran
TOF	(*S*)-Tetrahydro-5-oxo-2-furancarboxylic acid
TsOH	Tolyl alcohol
TSPI	1-N-(Toluenesulfonyl)prolyl isocyanate

1. Introduction

Asymmetric alcohols and their derivatives are ubiquitous, with numerous examples occurring as natural products and frequently as intermediates in the synthesis of chiral molecules. Chiral alcohols, being found in a variety of biogenic compounds and drugs such as the terpenoid family, steroids and insect pheromones, are often of great significance in the field of biomedical research.

Like other enantiomeric molecules such as amines, the antipodes of racemic alcohol drugs have substantial differences in their biological–pharmacological activities and their fates *in vivo*. For example, in the case of racemic warfarin, studies have shown that the hypothrombinemic effect of *S*-warfarin is approximately five to six times stronger than that of *R*-warfarin in both rat [1–3] and human [4]. Development of techniques for effective separation, sensitive detection and accurate determination of chiral alcohols is pressingly required in the areas of biomedical research for enantiomeric alcohols.

Chiral stationary phase (CSP), chiral mobile phase (CMP) and pre-column chiral derivatization (also called the indirect resolution mode) are the three major techniques that have been usually applied in the separation of chiral alcohols by HPLC. In recent years, considerable attention has been directed to the study of the applications of the CSP and CMP systems. However, the indirect resolution mode is still of great importance, especially for the biomedical analysis of alcohol drugs, because it has several advantages over the CSP and CMP methods:

(a) Better detectability of alcohols after derivatization. This feature is crucial for biomedical analysis because the weak UV absorption of the hydroxyl group of alcohol and the extremely low quantities of the analytes in biological samples make the conventional UV detection very unreliable.

(b) Employment of conventional LC systems. Achiral resolution systems used in the indirect resolution mode are more easily optimized than the chiral systems. They not only enable enantiomeric resolution in biological specimens containing complex components, but also provide good separation of other components.

(c) Achievement of better chemical and physical properties of alcohols to improve their chromatographic behavior.

(d) Confirmation of the analytical identity and selectivity for chiral alcohols in a complex matrix.

(e) Prevention of certain hydroxyl groups from decomposition during chromatography and detection of alcohol drugs.

Most derivatizing reactions involve a mechanism whereby the carboxylate anion or carbonyl group in the molecule of the derivatizing reagent is attacked by the negative uncharged group in the molecule to be analyzed (nucleophilic attack). Because the activity of uncharged nucleophiles decreases in the order N > O > S, chiral alcohols are usually more difficult to derive with CDRs than chiral amines. The CDRs that may be used for chiral alcohols are restricted to those with a high reactivity [5]. As a result, in contrast to the situation for the chiral amines, successful resolutions of optical active alcohols are rather more limited and have not been extensively reviewed as yet.

The present review intends to summarize and critically evaluate the methodology used to date and the application of chiral derivatizing reagents for the enantiomeric separation of alcohol drugs, based on the related literature published over the last two decades.

2. Classification and application of chiral derivatizing reagents for enantiomeric alcohols

Approximately fifty chiral derivatizing reagents have been developed thus far. Listed in Table 1 are the CDRs utilized for chiral alcohols, based on their functional groups, reaction conditions, resolution systems and applications along with the corresponding references. Seven types of chiral compounds are discerned: acids, activated acids, chloroformates, isocyanates, carbonyl nitriles, oxazolidin-2-ones and lactones. These reagents react with alcohols to form diastereomeric esters, carbamates and carbonates, as illustrated in Fig. 1.

3. Derivatization methodology of different chiral reagents

The methodologies for the reactions between the alcohols and the different CDRs will be discussed in this chapter, with respect to the molecular type of the CDRs.

3.1. Chiral acids

Diastereomeric esters can be prepared from acidic chiral reagents and alcohol enantiomers with the following methods: (a) The common esterification method under acidic conditions [9]. (b) Conversion of the acid into its acid chloride by the treatment of thionyl or oxalyl chloride freshly prepared for derivitizing with alcohols [10]. (c) Use of dicyclohexyl carbodimide (DCC) as the coupling reagent for the acid and imidazole as the catalyst for the reaction in organic

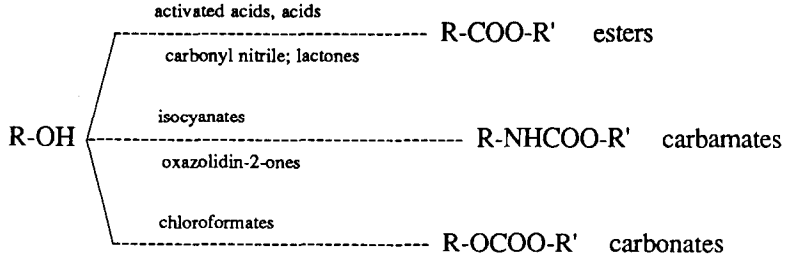
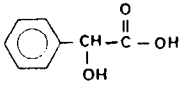
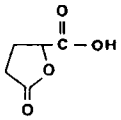
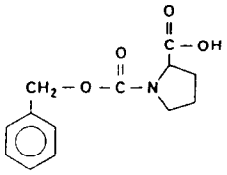
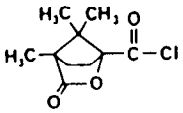
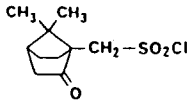
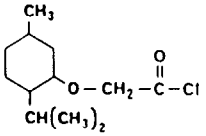
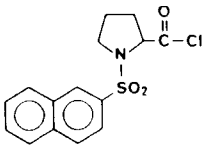
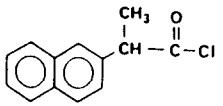
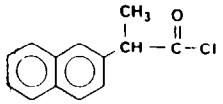


Fig. 1. Main types of derivatives formed from alcohols and the CDRs.

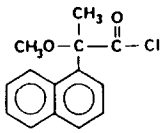
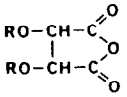
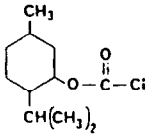
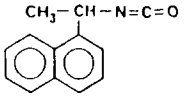
Table 1
Classification and application of chiral derivatizing reagents for alcohol enantiomers

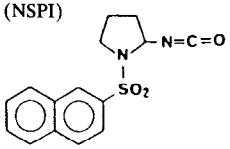
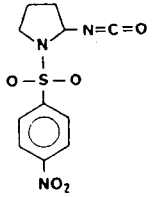
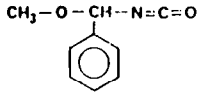
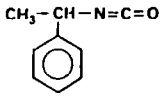
Reagents	Alcohol	LC system	Detection	Derivatizing procedure	Reference
Acids				*Esters*	
1. Mandelic acid 	alkyl alcohols, diols	NP/hexane, ethyl acetate	254	(a) TsOH, H_2O in benzene, refluxing for 4 h (b) At 0°C added DMAP and DCC, then 25°C for 22 h	9
2. (*S*)-Tetrahydro-5-oxo-2-furancarboxylic acid (TOF) 	alcohols	NP/ethyl acetate, hexane	254	Converted into its acid chlorides, then reacted with alcohols at r.t., overnight in the presence of pyridine	10
3. Carbobenzyloxy-L-proline (CBP) 	warfarin (in plasma)	NP/ethyl acetate, hexane, methanol, acetic acid	313	DCC, r.t., 14 h	11 12
Activated acids				*Ester*	
4. (−)-Camphanic acid chloride (CAC) 	dihydropyridine-based drug	NP/isooctane, acetonitrile, 2-propanol	229	Pyridine, r.t. 10 min	13
	proxyphylline	RP/sulphate buffer, methanol, acetonitrile, isopropanol	254	Pyridine	14
	proxyphilline	TLC/methanol, chloroform	254	Pyridine, r.t., 24 h	15
	proxyphilline (in plasma)	RP/methanol, H_2O	254	Pyridine, 20°C, 16 h	16

5. (−)-Camphorsulfonyl chloride (CSC) 	terfenadine	RP/methanol, H_2O, DMF, TEA	254, 280	Pyridine and DMF, r.t., 12 h, added DEA at r.t., 10 min prior to HPLC	17
6. Menthoxyacetyl chloride (MAC) 	dihydrobenzo[a]pyrene	NP/methylene chloride, ethyl acetate	254	added pyridine at 0°C, then r.t., 16 h	18 19 20
	Nipradilol	NP/hexane, ethyl acetate	254	pyridine, r.t., several hours	21
	benz[a]anthracene diols	NP/diethyl ether, cyclohexane		added pyridine at 0°C for 30 min., then r.t. overnight	22
7. *S*(−)-N-1-(2-Naphthylsulphonyl)-2-pyrrolidine carbonyl chloride (NSP-Cl) 	diltiazem	NP/chloroform, dichloromethane, methanol, diethylamine	254	pyridine, r.t., 15 min	23
8. D-2-(2-naphthyl)-propionyl chloride (NPC) 	diltiazem	RP/potassium ammonium acetate buffer,acetonitrile	254	pyridine, r.t., 15 min	24
9. *R*-(+)-1-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) 	B[a]Pdihydrodiols	NP/cyclohexane, diethyl ether	254	pyridine, r.t., 12 h	25 26 27
	dihydrodiols	RP/methanol, H_2O	254	pyridine, r.t., 12 h	28
	E/Z-10OH-AT (in human urine)	RP/perchloric acid, acetonitrile	254	pyridine, r.t., 12 h	29

Continued on p. 114.

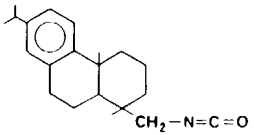
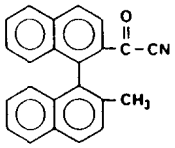
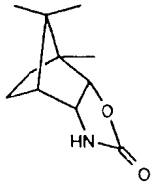
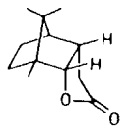
Table 1 *(continued)*

Reagents	Alcohol	LC system	Detection	Derivatizing procedure	Reference
10. (+)-6-Methoxy-α-methyl-2-naphthalene-acetyl chloride (MNC) 	cyclophosphamide (in plasma)	RP/potassium phosphate buffer, acetonitrile		added DMAP at 0°C for 2 h, then r.t., overnight	30
11. Tartaric acid derivatives 	amino alcohols	ammonium phosphate buffer, methanol	254	TCA and DCM, 40°C, 4 h	31
	propranolol	RP/aqueous acetic acid, acetonitrile (pH 4.0)	254	TCA, 50°C, 4 h	
	pyrrolidinol	RP/methanol, TEA, acetic acid buffer	254	TCA, 50°C, 4 h	33
Chloroformates				*Carbonates*	
12. Menthyl chloroformate (MCF) 	HETE	NP	UV	Pyridine, r.t., several hours	34
	warfarin	NP/heptane, ethyl acetate	UV 310	TEA, r.t., 30 min	35
	warfarin (in plasma)	NP/heptane, ethyl acetate	UV 310	TEA, r.t., 30 min	36
	benzhexol	RP/methanol, H_2O	280, 254	TEA and DMF, r.t., 2 h, added DEA for 10 min	17
	oxazepam dipropyllicum	RP/methanol, H_2O, THF, DMF, TEA	280 254	TEA, and DMF, r.t., 2 h added DEA for 10 min	17
Isocyanates				*Carbamates*	
13. *S* or *R*-1-(1-naphthyl) ethyl isocyanate (NEIC) 	1(1-naphthyl)-2,2,2-trifluoroethanol	Aluminum oxide/benzene	280	80°C, 65 h in toluene; or 80°C 10 h, catalyzed with DMEE or DBD	37
	methocarbamol (in biological fluids)	NP/hexane, isopropanol	280	85°C, 12 h, in ethyl acetate	38

	unsaturated hydroxy fatty acids	NP/hexane, ethyl acetate, THF	280	90–95°C, 1 h, under N2 in toluene	39
	diacyl glycerols	NP/propanol, hexane	280	PDPD, 50°C, overnight in toluene	40
	secondary alcohols	NP/cyclohexane, ethanol	275	363 K for 2–3 h.	41
	natural glycero derivatives	NP/hexane, ethyl acetate	254	DEA, 80°C, 36 h, in toluene	42
	oxazepam	RP/methanol, H_2O	254	80°C, 1 h, then r.t., overnight, in TEA and DMF; added DEA r.t., for 10 min	17
14. 1-N-(naphthylsulfonyl) prolyl isocyanate (NSPI) 	phenylpropanol	NP/light petroleum ether, isopropanol	254	80°C, 1 h, in toluene	43
15. 1-N-(toluenesulfonyl) prolyl isocyanate (TSPI) 	phenylpropanol	NP/light petroleum ether, isopropanol	254	100°C, 1 h, in toluene	44
16. α-Methoxybenzyl isocyanate (MBI) 	phenylpropanol	NP/light petroleum ether, isopropanol	254	60°C, 1 h, in toluene	45
17. *S*- or *R*-phenylethyl isocyanate (PEIC) 	misonidazole (plasma)	RP	UV		46

Continued on p. 116.

Table 1 *(continued)*

Reagents	Alcohol	LC system	Detection	Derivatizing procedure	Reference
18. Dehydroabietyl isocyanate (DBI) 	HETE	RP	254	DMA, in dichloromethylene reflux for 40 min	47 48 49
Carbonyl nitriles				*Esters*	
19. (+) or (−)-2-Methyl-1,1′-binaphthalene-2′-carbonyl nitrile (BNCN) 	alcohols	NP/pentane, ethyl acetate	FL (342, 420). (200pg)	60°C, 20 min, TEA	50
Oxazolodin-2-ones				*Esters*	
20. Oxazolidin-2-one 	trans-2-ethyl-cyclohexan-1-ol	NP/hexane, diethyl ether	258	Lawesson's reagent in toluene under reflux, then added the chloroformate of alcohols in DME at −78°C	51
Lactones				*Esters*	
21. Lactone (HTMB) 	alcohol	NP			52

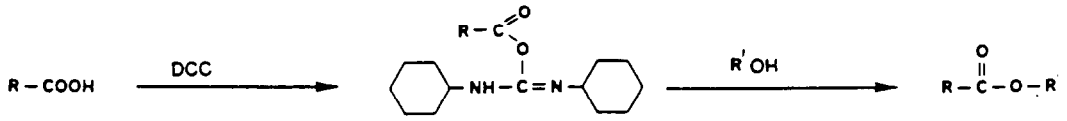


Fig. 2. The reaction mechanism for an alcohol and an acid in the presence of DCC.

solvents [6,7]. The reaction mechanism is shown in Fig. 2.

Banfield and Rowland [11,12] employed the reaction between warfarin and CBP in the presence of DCC and imidazole to analyze plasma samples and the experiments showed very satisfactory results.

Of the above methods, common esterification is hardly used anymore because of its laborious operation and low reactivity. Usually, the DCC method is highly reactive and it is therefore relatively widely applied. However, the acylation of sterically hindered secondary amines by the DCC method is usually ineffective [8]. The same problem might be anticipated for hindered secondary alcohols. Being readily available and chemical stable, acid CDRs are frequently utilized as precursors of the corresponding activated acid reagents.

3.2. Chiral activated acids

Activated acid reagents include carboxylic chlorides, sulfonyl chlorides and anhydrides. Derivatization of alcohols with these reagents usually requires the following conditions:

(a) Strictly anhydrous condition to secure a high yield of ester.

(b) The presence of a basic catalyst, such as pyridine, N,N-(dimethylamino) pyridine, 1-ethyl-3[3-(dimethylamino)propyl]carbodimide, triethylamine and N,N-dimethylaminoformamide. However, for tartaric acid anhydride reagents, an acid (trichloroacetic acid) is applied as the catalyst.

(c) In most cases, esterification is carried out at room temperature for several minutes. A prolonged reaction time is sometimes needed for certain alcohols in order to secure a high reaction yield.

Of the CDRs of chiral activated acids, CAC, CSC and MAC are widely used. They are readily synthesized from natural, optically pure compound (−)-camphor or (−)-menthol. As a result, these reagents often show a high enantiomeric purity and stable configuration, which benefit their enantioselectivity and detectability.

Application of CAC for the convenient and excellent resolution of four optical isomers of *R,S*-93522-004, a synthetic dihydropyridine-based calcium channel antagonist, has been studied by Ward and Manes [13]. The reaction mechanism and the chromatographic separation of the derivatives are shown in Figs. 3 and 4, respectively.

The sulfonyl structure of the CSC reagent endows its diastereomers with high detectability. Certain derivatives of CSC can be resolved in RP systems. For instance, racemic terfenadine was well separated with CSC in an RP system in our laboratory [17].

Owing to the bulky ring structure of their naphthyl or phenyl group, NSP-Cl, NPC, MNC and MTPA-Cl offer their derivatives high detectability and stereoselectivity. Two successful examples have been reported. Nusser *et al.* [29] have analysed E- and Z-10-hydroxyamitriptyline in human urine with MTPA-Cl on an RP system. The determination of cyclophosphamide enantiomers in plasma on an RP system after derivatization with MNC has been described by Reid *et al.* [30].

Acyl chlorides are highly reactive and are often used to derivatize sterically hindered secondary alcohols. Because of their limited long-term stabilities, acid chlorides are frequently freshly prepared by treating the corresponding carboxylic acid with thionyl or oxalyl chloride. Acyl chlorides are more susceptible to racemization than less reactive acyl agents. Racemization at a chiral carbon can be avoided by choosing reagents lacking an α-hydrogen atom, such as MTPA-Cl.

A special class of acylating reagents,

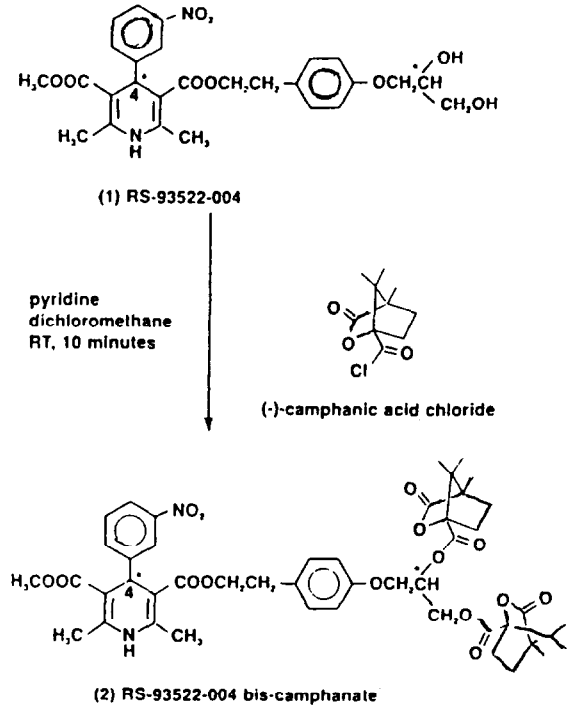


Fig. 3. Scheme of the derivatization of RS-93522-044, a synthetic dihydropyridine-based calcium channel antagonist, with (−)-camphanic acid chloride, RT = room temperature (reprinted from ref. 13 with permission).

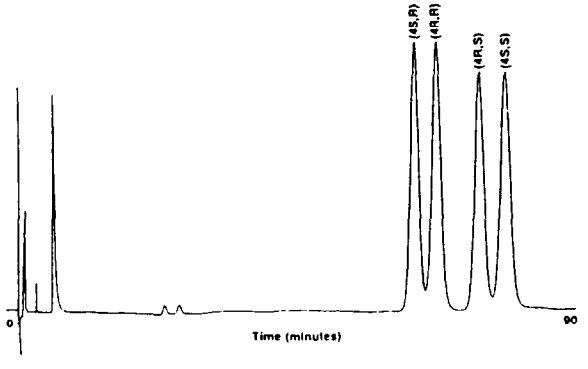


Fig. 4. HPLC separation of the RS-93522-004 bis-camphanate diastereomers (reprinted from ref. 13 with permission). (RS-93522-004 is a synthetic dihydropyridine-based calcium channel antagonist). Analytical column: Nucleosil silica (5 μm, 250 × 4.6 mm I.D.); mobile phase: 0.1% acetonitrile and 4% 2-propanol in isooctane (2,2,4-trimethylpentane); flow-rate: 2.0 ml/min; detection at 229 nm.

anhydrides of tartaric acid derivatives, are readily prepared from the natural compound, (−)-tartaric acid. They can only be used to resolve amino alcohol enantiomers by forming a ring structure, as illustrated in Fig. 5. Their sensitive detectability and good RP resolution could be attributed to their two benzoyl groups and the formation of a rigid intra-molecular ring [31–33].

3.3. Chiral chloroformates

Its high reactivity makes chiral chloroformate MCF an effective agent for the separation of chiral amines. However, only three applications for its use in alcohol isomer resolution have been reported. Under anhydrous conditions, MCF reacts with alcohols to produce carbonates in the presence of pyridine at room temperature in 30 min [34–36]. In order to expand the number of applications of the MCF reagent, several racemic alcohol drugs have been investigated in our laboratory. Among them, only benzhexol, oxazepam (shown in Fig. 6) and diprophyllincum yield satisfactory results [17].

According to our own experience, chiral chloroformates are quite promising for optical separation of alcohols. Their mild and convenient reaction conditions and the ability to resolve their derivatives by RP systems make the MCF agent very favorable for the analysis of optical alcohols in biological samples. However, more examples are still required to completely evaluate the enantioselectivity of MCF. Another agent in the group of chiral chloroformates, 9-fluorenylethyl chloroformate (FLEC), could also

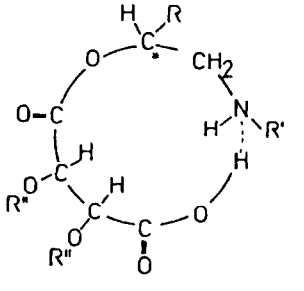


Fig. 5. The intra-molecular ring structure of the diastereomers formed from tartaric acid anhydride derivatives and amino alcohols (reprinted from ref. 6 with permission).

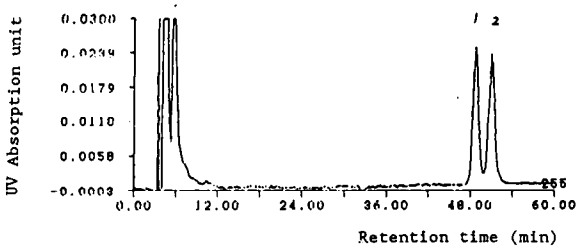


Fig. 6. Resolution of racemic oxazepam with MCF reagent. Peaks 1 and 2 represent the derivatives of racemic oxazepam. Column: Sarasep ODS 3 μm, 220 × 4.6 mm I.D.; mobile phase: methanol–H_2O–THF–DMF–TEA (52:26:14:7.6:0.4, v/v); flow-rate: 0.7 ml/min; detection at 255, 280, and 230 nm wavelength at the same time. The reaction solution is directly injected onto the LC system.

be potentially effective for the optical resolution of chiral alcohols. Theoretically, FLEC is considered to be superior to MCF because its bulky and rigid fluorenyl group may produce better enantioselectivity and UV detectability.

3.4. Chiral isocyanates

Chiral isocyanates, which are well-known for their excellent ability to separate enantiomeric amines, are also becoming valuable for the optical resolution of alcohols with good enantioseletivity and strong UV detectability. They react with alcohols when heated for several hours under anhydrous conditions to form diastereomeric carbamates. Usually, the reactions are catalyzed by alkaline compounds, such as N,N-dimethylethylethanolamine, di-*n*-butyltin diaurate, 4-pyrrolidinopyridine, triethylamine and N,N-dimethylaminoformamide. In the case of NEIC, the reaction temperature is often set at 80–90°C. A higher temperature (up to 110°C) often results in more by-products [38]. The main drawback of NEIC is the time-consuming process for derivatization. The reaction time depends not only on the alkaline condition and heating temperature, but also on the structural features of the alcohols. For example, unsaturated hydroxy fatty acid methyl esters can be derivatized with NEIC at 90–95°C in only one hour [39], while in the case of certain natural glycerol derivatives, the reaction requires 36 h at 80°C [42].

Recently, Severini *et al.* [38] have presented a satisfying application of NEIC for the analysis of methocarbamol enantiomers in biological fluids with a detection limit of 10 ng/ml. It is worthwhile to mention that NEIC has been used for the successful separation of racemic oxazepam on an RP system in our laboratory, the result of which is shown in Fig. 7 [17]. However, more examples of the resolution of racemic alcohols with RP separation systems after derivatization with NEIC are needed to evaluate the application of NEIC reagent.

In an effort to use chiral isocyanates as possible resolving agents for alcohols, three new chiral isocyanates, NSPI, TSPI and MBI, have been developed in our laboratory. Their reactions with racemic phenylpropanol require only one hour at 60 to 100°C [43–45]. One example is shown in Fig. 8. More studies are necessary to develop the application and commercialization of these reagents [43–45].

In addition, DBI has been found to be superior to both PEIC and NEIC for the sensitive determination and preparative resolution of hydroxyeicosatetraenoic acid (HETE) [47–49]. Also PEIC has been used for the HPLC separation of optically active alcohols [46].

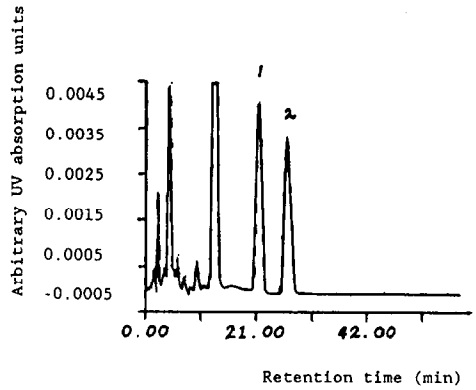


Fig. 7. Resolution of racemic oxazepam via derivatizing with NEIC. Peaks 1 and 2 represent the diastereomeric derivatives of racemic oxazepam. Column: Spherisorb 5 μm, 250 × 4.6 mm I.D.; flow-rate: 0.7 ml/min; detecting at 254, 280, and 230 nm at the same time; mobile phase: methanol–H_2O–THF–TEA (52:34:9.5:4.5, v/v). The derivatizing solution is directly chromatographed.

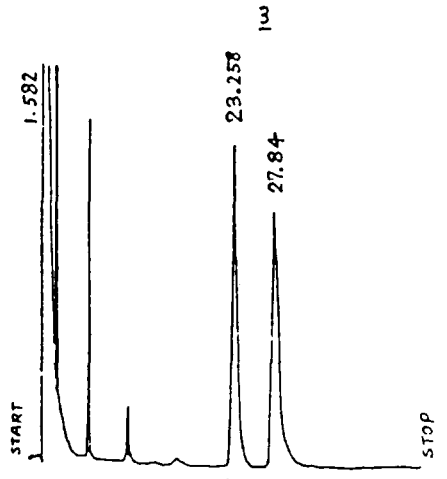


Fig. 8. Separation of the TSPI derivatives of racemic phenylpropanol (peaks at 23.26 min and 27.84 min). Column: silica gel 7–9 μm, 300 × 4 mm I.D.; detection at 254 nm; mobile phase: petroleum ether–isopropanol (99:1, v/v); flow-rate: 1.0 ml/min. The reaction solution is directly analysed (reprinted from ref. 44 with permission).

3.5. Chiral acyl nitriles

Acyl nitriles belong to a group of resolving reagents called axis compounds. An effective member of this group, BNCN, was introduced by Goto *et al.* in 1992 [50]. Quantitative coupling of alcohols with BNCN is achieved at 60°C for 20 min in the presence of triethylamine with the production of diastereomeric esters. The reaction mechanism is shown in Fig. 9.

The separation method with BNCN shows several features such as the use of mild reaction conditions, facile operation, good separation, and generation of highly fluorescent derivatives with a detection limit of 200 pg. The latter makes BNCN an ideal choice for the assay of specimens containing low quantities of enantiomers, since BNCN itself exhibits no fluorescence. Because of these properties BNCN would be particularly suited for the analysis of optical alcohols in

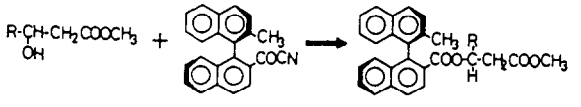


Fig. 9. The principle of the reaction between the BNCN reagent and alcohols (reprinted from ref. 50 with permission).

biomedical samples. However, no such examples have yet been presented.

3.6. Chiral oxazolidin-2-ones

A new homochiral reagent, oxazolidin-2-one (Fig. 10a), has recently been employed for the resolution of racemic alcohols [51]. The reaction mechanism is shown in Fig. 10.

Oxazolidinethione (Fig. 10b) is prepared by treating oxazolidin-2-one with Lawesson's reagent in toluene under reflux. The product is then allowed to react with the chloroformate of racemic trans-2-methylcyclohexan-1-ol in DME at −78°C to generate carbamate diastereomers with a separation factor of 1.34.

The following properties of oxazolidin-2-one favor its use in biomedical applications:

(a) Oxazolidin-2-one can be readily prepared in bulk quantity from inexpensive [(1*S*)-endo]-(−)-borneol.

(b) The thione group formed in the derivatization provides the derivatives with a necessary bathochromic shift and stronger UV absorption.

(c) The powerful topological bias inherent to the bornane skeleton benefits optical separation of its diastereomeric derivatives.

3.7. Chiral lactones

Chiral lactone was introduced by Noe in 1982 [52]. The enantioselectivity of the derivatives formed from lactone and racemic alcohols is exceptionally high. HTMB, a tricyclic lactone, is prepared from natural optically active camphor. The reaction principle is shown in Fig. 11.

3.8. Optimization of chiral derivatization

To accelerate and facilitate the completion of the reaction, several factors can be optimized, based on the principles of chemical kinetics and thermodynamics.

The absolute and relative amount of CDR and analytes

Concentrating the sample prior to derivatization may increase the reaction rate. The amount

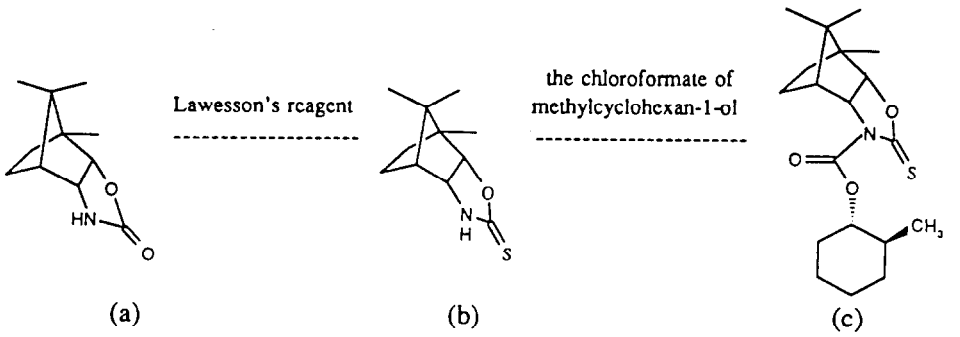


Fig. 10. The derivatization mechanism of alcohols with oxazolidin-2-one (reprinted from ref. 51 with permission).

of a CDR should be higher than that of the analytes in order to make the reaction equilibrium favor the formation of derivatives, but the stoichiometric ratio of CDR to chiral drugs should not be higher than 10. A higher ratio does not necessarily shorten the time needed for complete conversion; instead a larger excess of CDR may cause the formation of more side products and consequently complicate the analysis.

The reaction temperature

A reaction can usually be accelerated by a factor of 2 to 3 when the temperature is elevated by 10°C. The reaction temperature has to be evaluated to ensure optimal reaction rate and to minimize the formation of side products.

The reaction time

The formation of the derivative can be monitored at different time intervals during the reaction and the appropriate reaction time can be selected at that point in time where the peak area of the derivative reaches a plateau.

The presence of catalyst

Catalysts are usually necessary for the derivatization of alcohols. They may not interfere with the detection of derivatives, however. Because most derivatizations involve an acylation mechanism, basic compounds such as pyridine, alkylamines and their derivatives, are often used as catalysts due to their capability to accept the acid generated in the reaction.

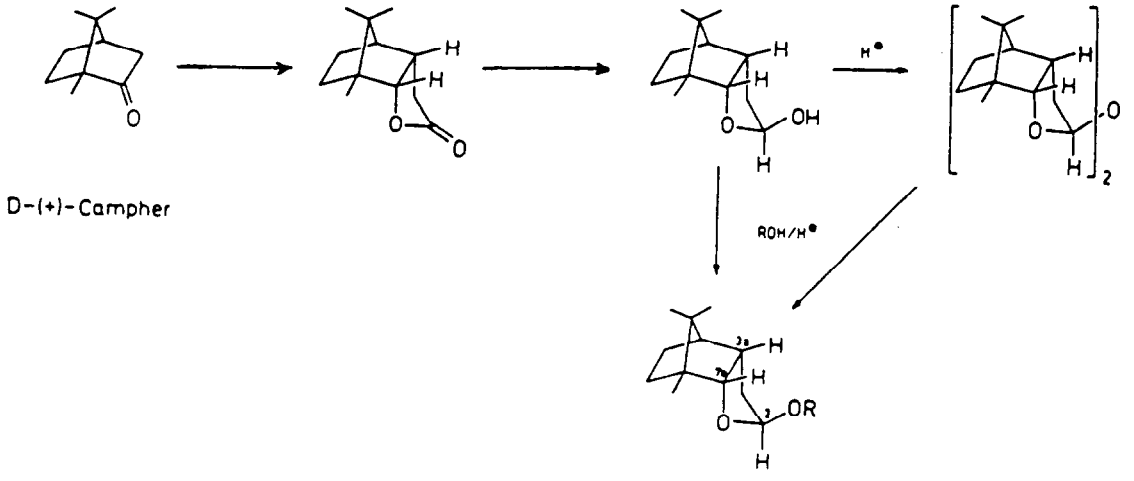


Fig. 11. The principle of the reaction between HTMB and an alcohol (reprinted from ref. 52 with permission).

The reaction solvent system

An ideal reaction solvent should have the following properties:

(1) provide the analytes with good solubility and optical stability;

(2) have no UV absorption at the detection wavelength or not interfere with the determination of the samples;

(3) facilitate the reaction, *e.g.* DMF and pyridine could serve as catalysts when used as solvents for the reaction;

(4) be compatible with the LC resolution system so that the reaction solution can be directly injected onto the chromatographic system. Usually, methanol and acetonitrile are compatible with RP-LC, while benzene, toluene, chloroform and dichloromethane are compatible with NP-LC. If the reaction solvent is not compatible with the LC system, it should be removed prior to the analysis. Evaporating the solvent under a stream of nitrogen at room temperature or under heating condition is the most convenient method for volatile reagents.

In the indirect separation mode, direct injection of the derivatization solution is often preferred in order to simplify the operations and reduce the loss of analytes. Several such examples have been presented [17,43–45]. In addition to the use of a compatible reaction solvent, excess CDR should be removed before the reaction solution is injected onto the LC system. This step is crucial for such reagents as chiral isocyanate, chloroformate and activated acid, since these are reactive to such a high degree that they may sometimes react with the supporting materials and reduce the column efficiency. An appropriate amount of N,N-diethylamine or ethanolamine is often added to the reaction solution at room temperature 10 min before LC analysis in order to remove excess CDR [17]. However, in selecting the reagent used to remove excess CDR, compounds such as 1,2-diaminoethane must be avoided. Its strong competition with alcohols, due to its high reactivity with CDR, may reduce the yield of diastereomeric derivatives of the chiral alcohols, according to our experience.

Several handbooks summarize the current information on the application of chiral reagents and they are also valuable in designing new reactions for chiral derivatization [53,54].

4. LC resolution system for diastereomeric derivatives of alcohols

The enantioselectivity of diastereomers depends on the structure of the chiral moiety of the CDR and the type of chemical bond formed in the reaction. A high conformational rigidity and a substantial difference in the physicochemical properties of the diastereoisomers are the two features that enhance the resolution of diastereomers.

The conformational rigidity of diastereomers depends on the following: a maximal distance of less than three atoms between the two chiral centers; bulky groups in the vicinity of the chiral centers of both the CDR and the analyte, such as naphthyl and phenyl groups; ring structures containing chiral carbon atoms, such as camphor, oxazolidin-2-one, chiral lactone HTMB, and DBTAA, which produce an intra-molecular binding ring after reaction with amino alcohols; polar or polarizable groups able to form hydrogen bonds or other intra-molecular interactions.

Substantial differences in physical and chemical properties, such as overall polarity, lipophilicity and adsorption on the surface of a stationary phase, are desirable for the separation of diastereomers. The differences depend on the polarity of the functional groups of the diastereomer molecule and the steric attraction or repulsion of the diastereomers to/from the surface of a stationary phase.

These two properties can explain the common phenomenon that an exceptionally good separation is more easily achieved for amide diastereomers than for esters. The amide bond has a planar backbone which is ideal for multiple-site binding to a polar adsorbent backbone. The size and the conformation of the substituents in the amide compound directly influence its degree of binding to a polar surface. This sterically controlled adsorption mechanism directly correlates with the differences in retention behaviour of the

diastereomers. In the case of diastereomeric esters, however, hydrogen bonding and polar dipole–dipole interactions at the surface are minimal. Rotation around an ester bond is not significantly hindered. To obtain considerable resolution of esters, bulky groups such as naphthyl, phenyl and camphor in CDRs are desired. Other diastereomeric compounds, such as carbamates, lactams and oxazolidones, also show better separation than their corresponding esters because of their amide structures.

In general, separation in nonaqueous chromatographic systems is predominantly based on polar interactions between sample and stationary phase. Most diastereomers obtained from alcohols, particularly diastereomeric esters, are commonly resolved in normal-phase LC systems, based on difference in their polar interaction with the stationary phase. Mixtures of ether and cyclohexane are often superior to other aprotic solvents for the resolution.

In a reversed-phase system, separation is based on the hydrophobic difference of the diastereomers. Only a few successful applications of RP systems for the optical separation of alcohols have been reported [14,16,17,29,30,46]. These successful resolutions may be explained by the fact that the size, the polarity and the conformation of the alcohols to be analysed also greatly influence the LC behavior of their derivatives. Although these factors do not directly determine the stereoselectability of the diastereomers, they sometimes cause the suitable retention of their derivatives on the RP systems.

5. Detectability of diastereomers formed from alcohol enantiomers

CDRs should endow their derivatives with not only good enantioselectivity but also high detectability. The following structural features of CDRs and their resulting derivatives are desirable: the presence of a naphthyl or phenyl group in the CDR (may be detected by UV absorption at 254 or 280 nm or by fluorescence emission between 320 and 350 nm); formation of structures such as carbamate, carbonate, sulfonyl acid ester, lactam or oxazolidone during derivatization; and the presence of N, S, F, or Cl atoms in the CDR.

NEIC is usually considered to be one of the most effective CDRs for improvement of the detectability of alcohols. NEIC-derivatives are in most cases fluorescent (ex/em = 285/330 nm), which makes NEIC a sensitive reagent for the analysis of low concentration samples such as biomedical specimens.

When enantiomers that have intrinsic fluorescence or strong UV absorption are to be assayed at low concentrations in a sample, it may be advantageous to use so-called transparent reagents, *e.g.* BNCN [50]. Such reagents and their degradation products do not themselves absorb light at the wavelength used for the sample, nor do they emit any fluorescence. These properties would eliminate the problem of introducing fluorophores or chromophores into the system in order to reduce the detection limit for the sample.

6. Conclusions and future trends

In certain cases, a pair of diastereomeric derivatives of a racemic drug may give different peak areas in the resulting chromatogram. This phenomenon may result from the limitations of the indirect resolution method discussed below:

(a) The two enantiomers of a racemate may have different reaction rates. In this case, excess CDR, longer reaction time and higher reaction temperature should be employed. A different CDR should be selected if the above adjustments fail to improve the chromatographic result.

(b) The pair of diastereomeric derivatives of enantiomeric alcohols may have different UV detectability. This phenomenon can be confirmed if the two UV curves of the diastereomers do not overlap entirely when a three-dimension UV detector is used to determine the UV absorption of a pair of diastereomers simultaneously. If such is the case, a homochiral compound, *i.e.* the (*R*)- or (*S*)-isomer of the chiral alcohol, or a sample containing the chiral alcohol with a

known optical purity, must be used to correct the experimental results. For example, with the same derivatization and LC system, the chirally pure (*R*)-isomer of the optical alcohol can be used to make a standard curve that indicates the relationship between the concentration of the (*R*)-isomer and its peak area. Then the amount of the (*R*)-isomer of the chiral alcohol in the sample can be calculated from the standard curve.

(c) Optical impurities in a CDR often are considered to be the main limiting factor for the accuracy of the determination in the indirect resolution mode. CDRs may be purified by chromatographic as well as by classical non-chromatographic techniques. The optical purity of a CDR must be known before use or determined by derivatizing with an optically pure substance or by chiral chromatography. The actual amount of an isomer ($R_{sa,theo}$ and $S_{sa,theo}$) in a sample can be calculated with the following equation:

$$\left[\left(R_{sa,theo} \cdot S'_{CDR}\right) + \left(100 - R_{sa,theo} \cdot R'_{CDR}\right)\right] \cdot 0.01 = R_{sa,exp}$$

where $R_{sa,theo}$ is the actual value of the (*R*)-enantiomer of the analyte, $R_{sa,exp}$ is the experimental value of the (*R*)-enantiomer of the analyte, S'_{CDR} is the percentage of the (*S*)-enantiomer of the CDR, R'_{CDR} is the percentage of the (*R*)-isomer as an impurity in the CDR of the (*S*)-isomer ($S'_{CDR} + R'_{CDR} = 100$).

For instance, a CDR containing 99 parts of (*S*)-isomer and 1 part of (*R*)-isomer is applied to determine a sample. If the $R_{sa,exp} = 98$, $R_{sa,theo}$ will be 99, thus $S_{sa,theo}$ should be 1.

Most commercialized CDRs have an optical purity of *ca.* 99%. Because *R*/*S* ratios of 50:50 to 95:5 are common in biomedical samples, a one percent error resulting from the impurity of the CDR can usually be neglected. Meanwhile, the features of the indirect resolution mode that improve the detectability and selectivity for optical alcohols are crucial for biological samples which are usually difficult to analyse, because they contain only a low amount of drugs together with a large amount of other complex components. Therefore indirect resolution methods are often superior to other methods for use in biomedical applications.

In the design of a method using the indirect resolution mode, the following aspects should be taken into account:

–choosing a CDR and quantifying its optical purity;

–identifying the structures of the diastereomeric derivatives;

–selecting the catalyst and solvents for the reaction;

–optimizing the reaction temperature and time;

–adjusting the absolute and relative concentrations of the CDR and the analytes;

–removing the excess CDR and those reaction solvents that are incompatible with LC systems, or extracting the diastereomeric derivatives prior to LC analysis;

–examining the reproducibility of the reaction;

–correcting the experimental results by using the optical purity of the CDR and a homochiral compound such as the (*R*)- or (*S*)-isomer of the alcohol to be resolved, or a sample containing the optical alcohols with a known optical purity.

Whenever a drug has a simple structure and a hydroxyl group connected with or near a chiral center, an indirect separation method is often less costly, more straightforward and practical than CSP and CMP modes. Besides, in the indirect resolution mode more easily optimized chromatographic systems are available to achieve a better separation among the diastereomeric derivatives of alcohol drugs and other components in biological samples than in the direct resolution mode.

More than twenty CDRs have been developed for alcohols. Chiral acid chloride, isocyanate and chloroformate are most commonly used. Because of the diversity of the groups with respect to their type, size, polarity and spatial arrangement around their chiral centers, various chiral alcohols show substantial differences in their LC properties and their reactivities with the different CDRs. Whether an indirect resolution

approach is successful or not depends largely on the choice of the CDR, the derivatization conditions and the LC separation system. A similar thoughtful selection should also be made when choosing the CSPs and the mobile phases in the direct resolution mode.

The indirect separation techniques for chiral alcohols still need further investigation, especially with respect to the following items:

(a) developing ideal CDRs of high optical purity, chemical stability, reactivity, enantioselectivity and detectability;

(b) optimizing derivatization conditions and LC resolution systems, especially the RP systems, for the existing CDRs;

(c) expanding the use of indirect separation techniques for the resolution of chiral alcohols in biomedical samples;

(d) developing simple and rapid reaction applicable in automated pre-column derivatization.

Because of the great significance of chiral alcohols in biomedical research and because of the difficulties encountered in the resolution of enantiomeric alcohols due to their low reactivities with CDRs and less hindered rotation of their ester derivatives compared to that of the amide derivatives of chiral amines, the separation of optical alcohols is generally considered to be crucial and challenging and is attracting more and more attention not only in the field of biomedical analysis, but also in the research on chiral LC separation methodology. Commonly, for the resolution of chiral amines and acids, a CSP method is the first choice. But for chiral alcohols, the indirect resolution method is still very important, especially in the case of the analysis of alcohol enantiomers in biomedical samples, owing to its advantages over the CSP and CMP methods.

In conclusion, the indirect separation mode has been playing an important role in the resolution of chiral alcohols. With the improvement of the methodology, the introduction of sample processors and the employment of robotic systems, this technique will become increasingly popular, especially in the field of biomedical analysis.

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