Resolution of Chiral Interconvertible Diastereoisomers of a 2,18-Bridged Biliverdin Mediated by First-order Asymmetric Transformation

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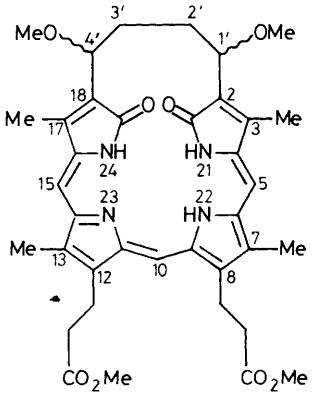
Chiral diastereoisomers of the bridged biliverdin 2,18-(1′,4′-dimethoxybutane-1′,4′-diyl)-8,12-bis(2″-methoxycarbonylethyl)-3,7,13,17-tetramethylbilin-1,19-(21*H*,24*H*)-dione *rac*-(**1a**) and *rac*-(**1b**) have been resolved and isolated from the four-component mixture. Repetitive enrichment of *M*-helical isomers with (*R*)-(−)-mandelic acid followed by chromatographic separation afforded one enantiomer of each diastereoisomeric pair, *viz.* (*M*-*SS*)-(**1a**) and (*M*-*RR*)-(**1b**). The corresponding enantiomorphic species (*P*-*RR*)-(**1a**) and (*P*-*SS*)-(**1b**) were each obtained from their respective diastereoisomeric counterpart by thermal conversion. The enantiomeric purities achieved (>95%) were checked by n.m.r. techniques. The rotational strengths *R* (absolute values) of the visible c.d. bands exhibited by the diastereoisomers of (**1**) are close to one another and similar to those reported for open-chain bilatrienes. The influence of chirality centres on helical excess (h.e.) and helicity of the bridged bilatriene moiety and its pronounced solvent dependence is discussed.

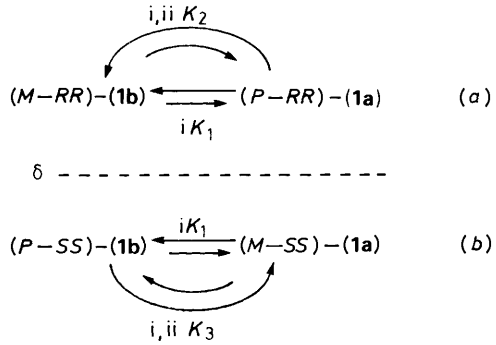
Biliverdins in the *Z*,*Z*,*Z*-configuration, like hexahelicene adopt a non-planar, helical arrangement in order to avoid mutual interference of the pyrrolinone rings.[1,2] Hexahelicene may be easily resolved by classical methods and its enantiomers exhibit considerable optical activity due to the inherent chirality of the chromophore.[3] As expected, the magnitudes of chiroptical properties of the bilatriene helix are also large.[4] However, interconversion barriers are low for non-bridged derivatives[5] so that a helical excess (h.e.)† can only be maintained as long as the discriminating environment persists. This kind of resolution can also be mediated inter- and intra-molecularly. While the h.e.s induced by chiral additives[1,6,7] are less pronounced, large discriminations (even approaching 100% h.e.) have been reported for bilipeptides containing appropriate amino acid sequences.[4,8]

Recently we described the synthesis of the bridged biliverdins *rac*-(**1a**) and *rac*-(**1b**) which adopt fixed helical conformations.[9] These compounds are chiral and diastereoisomeric to one



(**1**)

another, each possessing three chirality elements: two homochiral centres (1′-*R*, 4′-*R*) or (1′-*S*, 4′-*S*)‡ and the bilatriene backbone of *M* or *P* helicity. Owing to the mobility of the bilatriene helix, the thermally induced isomerisation *rac*-(**1b**) ⇌ *rac*-(**1a**) takes place at ambient temperatures (Scheme).



Scheme. Equilibria (*a*) and (*b*) involved in isomerisation and asymmetric transformation of the four stereoisomers of (**1**) in benzene. *Conditions and reagents:* i, thermal energy; ii, optically active acid; length of arrows qualitatively refers to equilibrium positions in benzene prior to (K_1) and after addition of (*R*)-(−)-mandelic acid (bent arrows, $K_2 \neq K_3$).

However barriers to helix inversion [$\Delta G^{\ddagger}_{(1a)}$ *ca.* 89 kJ mol^{-1} and $\Delta G^{\ddagger}_{(1b)}$ *ca.* 87 kJ mol^{-1} at 293 K in ethanol][10] are almost twice as large as those reported for non-bridged biliverdins, so that the diastereoisomers *rac*-(**1a**) and *rac*-(**1b**) may easily be separated by t.l.c. at moderately low temperatures. Thus, under these conditions the four stereoisomers of (**1**), if isolated as single compounds, can be expected to be resistant towards isomerisation even without the maintenance of the chiral surrounding that is the resolving agent.

Due to the basicity of the pyrrolenine nitrogen incorporated in the helical framework, resolution of compounds (**1**) with optically active acids seemed promising. However, conventional methods, *e.g.* recrystallisation of diastereoisomeric salts with optically active acids would require different and time-intensive handling to be performed at relatively low temperatures. A

† This notation refers to helicity only and omits any additional chirality elements. Thus it is applicable to both enantiomers *and* diastereoisomers. In a similar sense, 'centrochiral excess' (c.e.) is used for configurational homogeneity with respect to chirality centres. 'Enantiomeric excess' (e.e.) will then be reserved exclusively for antipodes.

‡ In the following the numbering will be omitted.

further complication would arise from the presence of four diastereoisomeric species. A kinetic method has been applied[11] to resolution of a racemic *N*-21,*N*-24 methylene-bridged biliverdin but afforded low optical yields (*ca.* 5%).[11] Moreover problems similar to those mentioned above are expected to arise if the same method is used in the present case. Bearing in mind the impracticability of these approaches it was our goal to derive a more convenient method for resolution and separation.

In addition, this report evaluates the chiroptical properties of the bilatriene *M* and *P* helix and the influence of the chirality centres positioned in the four-membered bridge on both the helicity and the h.e.

Results and Discussion

Our strategy for the isolation of the four stereoisomers from the mixture of (**1**) was based on first-order asymmetric transformation*[12,13] and comprises three steps: (*i*) enrichment of isomers of *M*-helicity, (*M*-*SS*)-(**1a**) and (*M*-*RR*)-(**1b**), (*ii*) their isolation, and (*iii*) their respective conversion into (*P*-*SS*)-(**1b**) and (*P*-*RR*)-(**1a**). The preferred helicity obtained thereby follows from theoretical calculation,[14] correlating *M*-helicity with a negative and positive Cotton effect of the visible and first u.v. band, respectively. The concept used is outlined first.

If the isomerisation *rac*-(**1b**) $\rightleftharpoons$ *rac*-(**1a**) takes place, two independent enantiomeric processes [Scheme (*a*) and (*b*)] are involved. Their equilibrium constants K_1 are equal as long as the environment is achiral but become different (K_2, K_3) if a chiral influence is exerted on the equilibria. (*R*)-(−)-mandelic acid discriminates between *P*- and *M*-helices, stabilising the latter. Consequently, at ambient temperatures the equilibria (*a*) and (*b*) are shifted to opposite sides and the populations of (*M*-*SS*)-(**1a**) and (*M*-*RR*)-(**1b**) thus increase at the expense of the *P*-helical isomers. At −15 °C diastereoisomers exhibit kinetic stability and a chromatographic separation (t.l.c.) can be performed. Further enrichment up to e.e. >95% can be achieved if this procedure is repeatedly applied. Accordingly, each main fraction was separately treated with (*R*)-(−)-mandelic acid at room temperature, cooled and subjected to t.l.c. The enantiomeric excess after the *n*th enrichment step and equilibrium constants K_2 and K_3 are related by equation (1) where $p = 1/K_2 + 1$ and $q = 1/K_3 + 1$ for the fraction

$$\text{e.e.} = \frac{p^n - q^n}{p^n + q^n} \qquad (1)$$

enriched in (*M*-*SS*)-(**1a**), and $p = K_3 + 1$ and $q = K_2 + 1$ for the fraction enriched in (*M*-*RR*)-(**1b**). The other two stereoisomers (*P*-*SS*)-(**1b**) and (*P*-*RR*)-(**1a**) are then obtained from (*M*-*SS*)-(**1a**) *via* process (*b*) and from (*M*-*RR*)-(**1b**) *via* process (*a*), respectively, by equilibration in achiral solvents at room temperature followed by t.l.c. at −15 °C. Since the equilibrium constant K_1, necessarily equal for both processes, critically depends on the solvent, the extent of conversion can be optimised (see ref. 9).

For isolation of isomers with *M*-helicity (*R*)-(−)-mandelic acid was added to (**1**) dissolved in benzene at 20 °C. The c.d. spectrum of the bilatriene chromophore monitored at λ *ca.* 650 nm increased and the maximum value (Δε = −72)† was attained when three equivalents were present. Further addition was limited by the solubility of (*R*)-(−)-mandelic acid. The phenomena observed by c.d. are due to the formation of an excess of stereoisomers of *M*-helicity over those of *P*-helicity, both of which are of equal population in the absence of the chiral additive. More precisely, equilibria (*a*) and (*b*) are shifted to opposite sides, as indicated by the bent arrows in the Scheme, to afford an excess of (*M*-*SS*)-(**1a**) and (*M*-*RR*)-(**1b**). Changing the solvent from benzene to chloroform strongly diminishes the h.e. and in ethanol no discrimination occurs at all. Apparently more polar or protic solvents compete strongly with the discriminating forces built up with chiral solutes, this situation being typical for first-order asymmetric transformations.[12] The discriminatory efficiency of optically active agents other than (*R*)-(−)-mandelic acid [(*S*)-(−)-chloropropionic acid, (*R*)-(−)-2-phenylbutyric acid, (*S*)-(+)-lactic acid, and (*1S*)-(+)-camphorsulphonic acid] are lower by factors of 2–8.

At this point it seems appropriate to make some general remarks on the protonation of biliverdins. This process has been shown to be very complex for non-bridged derivatives, involving differently charged species and aggregates adopting different conformations, and is associated with striking changes in u.v.–vis spectra.[15] In the case of compounds (**1**) differences in the u.v.–vis spectra observed during titration with optically active acids in benzene are small. However, even if non-bridged biliverdins are considered, no substantial changes in the u.v.–vis spectra occur on addition of 3 equivalents of (*R*)-(−)-mandelic acid using the conditions under which chiral discrimination takes place.[16] At the present time we are therefore unable to discern whether salt formation occurs at all and, if so, which of the great variety of protonated species possible are involved in helix discrimination.

The ^{1}H n.m.r. spectrum of the equilibrated mixture of (**1**) and 3 equivalents (*R*)-(−)-mandelic acid in benzene at room temperature displays signals from four stereoisomeric species in different proportions corresponding to (*M*-*SS*)-(**1a**), (*P*-*RR*)-(**1a**), (*M*-*RR*)-(**1b**), and (*P*-*SS*)-(**1b**). Especially the signals from *meso* methine protons attached to C-5 (C-15) at *ca.* 6.3 ppm (Figure 1) and to C-10 at *ca.* 7.6 ppm (not shown) are well separated, providing the basis for quantitative evaluations. The assignment of individual signals to the corresponding isomers follows from (*i*) the restriction (2) which holds true, irrespective

$$x_{(M\text{-}SS)\text{-}(\mathbf{1a})} + x_{(P\text{-}SS)\text{-}(\mathbf{1b})} = x_{(P\text{-}RR)\text{-}(\mathbf{1a})} + x_{(M\text{-}RR)\text{-}(\mathbf{1b})} \qquad (2)$$

of the h.e. generated from the unresolved mixture (**1**); (*ii*) from the predominance of species of *M*-helicity, and (*iii*) from the ratio of diastereoisomers $x_{(\mathbf{1a})}/x_{(\mathbf{1b})}$ computed from the corresponding n.m.r. signals of the (poorly resolved) methine protons at C-1′ and C-4′ [*ca.* 4.0 (**1a**) and 4.7 (**1b**) ppm]. Hence h.e. [Equation (3)] can now be evaluated to be 54%. Since

$$\text{h.e.} = \frac{x_{(M\text{-}SS)\text{-}(\mathbf{1a})} + x_{(M\text{-}RR)\text{-}(\mathbf{1b})} - x_{(P\text{-}RR)\text{-}(\mathbf{1a})} - x_{(P\text{-}SS)\text{-}(\mathbf{1b})}}{x_{(M\text{-}SS)\text{-}(\mathbf{1a})} + x_{(M\text{-}RR)\text{-}(\mathbf{1b})} + x_{(P\text{-}RR)\text{-}(\mathbf{1b})} + x_{(P\text{-}SS)\text{-}(\mathbf{1b})}}$$

u.v.–vis absorption spectra of diastereoisomers differ slightly (see ref. 9 and below) the same can be expected for the c.d. spectra. Therefore the c.d. spectrum of this mixture obtained at low temperatures after neutralisation of the discriminating (*R*)-(−)-mandelic acid comprises an envelope of two similar but distinctly different bisignate curves from (*M*-*SS*)-(**1a**) and (*M*-*RR*)-(**1b**). Nevertheless the values obtained for the two main bands [Δε (λ *ca.* 650 nm) = −130 ± 10 and Δε (λ *ca.* 380 nm) = +195 ± 10 for h.e. = 100%] reflect the essential characteristics of the bilatriene *M*-helix of (**1**) (see below).

From Figure 1(*b*) further important information can be extracted with respect to enantiomeric purities (e.e.) of (*M*-*SS*)-

* Alternatively referred to as asymmetric transformation of the first kind.[12]

† This value does not differ essentially from that obtained immediately after neutralisation of the resolving agent.

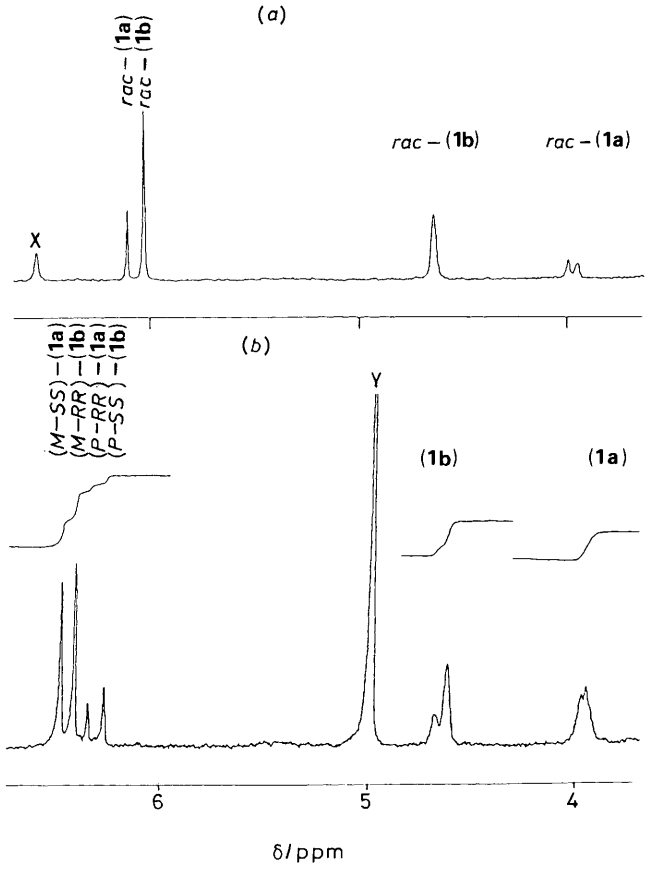


Figure 1. ^{1}H N.m.r. spectra (250 MHz; 20 °C) of equilibrated mixtures of (**1**) (*a*) of *ca.* 1 × 10^{-2} mol dm^{-3} solution in benzene and (*b*) after addition of 3 equivalents of (*R*)-(−)-mandelic acid. Signal X (*a*) is due to protons at the pyrrolinone nitrogens of *rac*-(**1a**) while Y (*b*) refers to the methine H (at C-2) of (*R*)-(−)-mandelic acid.

Table 1. Repetitive enrichment of (*M-SS*)-(**1a**) and (*M-RR*)-(**1b**) starting from (**1**) (12 mg); yield and e.e.[a] after the *n*th cycle refer to the respective main fractions.

	(*M-SS*)-(**1a**)			(*M-RR*)-(**1b**)	
n	e.e. (%)	yield/mg	*n*	e.e. (%)	yield/mg
1	52	4.5	1	47	4.7
2	80	2.7	2	78	2.1
3	93	1.7	3	91	1.2
4	>95	1.0	4	>95	0.6

[a] Determined by n.m.r. and/or c.d. spectroscopy.

(**1a**) (e.e. *ca.* 60%) and (*M-RR*)-(**1b**) (e.e. *ca.* 50%)* to be expected after chromatographic separation (see below). From an estimate of the constants K_2 *ca.* 0.23 and K_3 *ca.* 2.8 it follows from equation (1) that stereoisomeric homogeneity (e.e. >95%) will require four enrichment cycles (n = 4). Following these expectations the equilibrated mixture of (**1**) with (*R*)-(−)-mandelic acid was subjected to t.l.c. at −15 °C. By this procedure the auxiliary acid was simultaneously stripped off. The e.e. values obtained for the respective main fractions of (*M-SS*)-(**1a**) and (*M-RR*)-(**1b**) after the first (n = 1) and the following three enrichment cycles are contained in Table 1.

* These values do not completely conform with those obtained after separation by t.l.c. (Table 1, n = 1). This is due to slight (*ca.* 5%) reconversion occurring during application to t.l.c. plates at temperatures close to the m.p. of benzene (*ca.* 5 °C).

Table 2. C.d.[a,b] [$\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm); R/erg cm^3] and electronic absorption spectra[b] [ε_{max}/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm); D/erg cm^3] of the two enantiomeric pairs of diastereoisomers of (**1**) and the bilipeptide (**2**)[c] for 5 × 10^{-6} mol dm^{-3} solutions in ethanol at 3 °C.

	c.d.		u.v.–vis	
	Δε (λ)	R × 10^{38}	ε (λ)	D × 10^{35}
(*M-SS*)-(**1a**)	−140.8 (653)	−4.5		
	+214.2 (385)	+7.1	16 100 (662)	2.7
			46 300 (385)	6.9
(*P-RR*)-(**1a**)	+137.5 (653)	+4.4		
	−207.9 (385)	−7.0		
(*M-RR*)-(**1b**)	−127.3 (652)	−4.5		
	+214.8 (370)	+7.1	14 500 (662)	2.7
			46 400 (373)	7.0
(*P-SS*)-(**1b**)	+125.8 (652)	+4.5		
	−212.4 (370)	−7.1		
(**2**)	+108.8 (661)	+4.3	13 100 (661)	2.7
	−149.1 (378)	−5.1	46 700 (379)	7.5

[a] Differences in magnitudes observed for antipodes are due to experimental fluctuations; e.e. >95%; values are corrected for slight isomerisation during recordings. [b] Error ±5%. [c] Since the h.e. of (**2**) in ethanol is low, chloroform or benzene (in which complete chiral discrimination takes place) must be considered. In general biliverdins in chloroform and ethanol exhibit similar u.v.–vis spectra indicating great similarities in the helix geometries. Therefore the values taken from ref. 4 refer to chloroform solutions (3 × 10^{-5} mol dm^{-3}, at 20 °C).

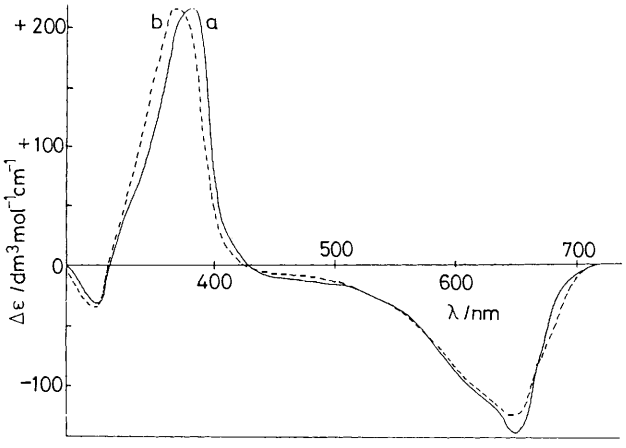
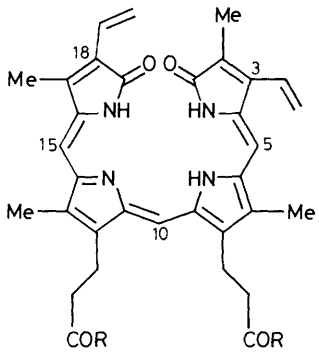


Figure 2. C.d. spectra of diastereoisomers (*a*) (*M-SS*)-(**1a**) and (*b*) (*M-RR*)-(**1b**) for 5 × 10^{-6} mol dm^{-3} solutions at 3 °C in ethanol; e.e. >95%; spectra are corrected for slight isomerisation during recordings; see also Table 2.

The corresponding enantiomorphic entities (*P-RR*)-(**1a**) and (*P-SS*)-(**1b**) were generated from (*M-RR*)-(**1b**) and (*M-SS*)-(**1a**), respectively, through equilibration at room temperature [Scheme; equilibria (*a*) and (*b*)] followed by t.l.c. at −15 °C. Since the constant K_1 is strongly solvent dependent (see below) large conversions (up to 80%) are achieved if equilibration is performed in appropriate media prior to t.l.c. Thus, (*P-SS*)-(**1b**) was obtained in large yield (70%) if (*M-SS*)-(**1a**) was equilibrated in carbon tetrachloride. Similarly, conversion of (*M-RR*)-(**1b**) to (*P-RR*)-(**1a**) through equilibrium (*a*) was favourably performed in dichloromethane.

The c.d. spectrum and electronic absorption parameters (ethanol, 3 °C) of the four optically pure stereoisomers are given in Table 2. For comparison of band shapes, c.d. spectra in the range from λ = 800–280 nm for one diastereoisomeric pair



(2)

R=(S)-Val-(S)-Pro-(S)-Ala-(S)-Val-OMe

Table 3. Isomeric distribution (%)[a] of (*P-RR*)-(**1a**) and (*M-RR*)-(**1b**) [equilibrium (*a*)][b] in various solvents as determined by c.d. $\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1} (λ_{max}/nm)[c] and corresponding ratios of dipole strengths $f = D$(u.v.)/D(vis.) of electronic absorption bands for *ca.* 5 × 10^{-5} mol dm^{-3} solutions at 20 °C.

	Δε (λ)	(*P-RR*)-(**1a**)	(*M-RR*)-(**1b**)	*f*
Dichloromethane	+85.6 (650) −128.7 (388)	80	20	2.7
Chloroform	+64.6 (652) −104.7 (389)	73	27	2.7
Ethanol	+60.7 (652) −95.0 (388)	71	29	2.7
HMPA	−34.6 (661) −74.2 (370)	34	66	2.4
Benzene	−43.1 (652) +79.0 (370)	31	69	2.4
Tetrahydrofuran	−54.3 (654) +98.8 (369)	27	73	2.5
Carbon tetrachloride	−82.0 (652) +131.0 (372)	17	83	2.5

[a] Mean values obtained from c.d. at λ *c.a.* 540 and *ca.* 377 nm, error ±3%. In addition use of the $\Delta\varepsilon_{max}$ values at λ *ca.* 650 nm provides satisfactory accuracy for evaluation since the positions of the maxima of (**1a**) and (**1b**) species are close to one another in contrast with the first u.v. band. [b] The same results were obtained from equilibrium (*b*). [c] Equilibria were reached within 30 min; c.e. >95%.

[(*M-SS*)-(**1a**) and (*M-RR*)-(**1b**)] are additionally displayed in Figure 2. Positions and shapes for the two main bands of (**1a**)- and (**1b**)-type species parallel deviations in the u.v.–vis spectra. Δε values at λ *ca.* 650 nm of (**1a**) and (**1b**) components differ by *ca.* 10%. However, if rotational strengths (*R*) are compared, making an allowance for the different band widths, excellent agreement is obtained for both the visible and u.v. bands. The *R* values of (*P-RR*)-(**1a**) and (*P-SS*)-(**1b**) of the visible bands are close to those reported for the non-bridged biliverdin-IXα *P*-helix generated *via* intramolecular peptide-mediated chiral discrimination[4] in compounds such as (**2**) (Table 2). In contrast appreciable deviations in u.v.–c.d. bands of (**1**) and (**2**) occur. However, from c.d. data of bilatrienes which have been accumulated in recent years[4,8,17] it can be concluded that this band is not only influenced by helix geometry, as found for the visible band, but is also susceptible to the substitution pattern and most probably to helix mobility. Hence, the rotational strength (*R*) obtained for the visible c.d. band reflects a more general property of the helical bilatriene backbone, and is not restricted to one single compound. In this respect, the *R* values of the band at λ *ca.* 380 nm seem less valuable and may change from compound to compound.

The c.d. spectra of (*M-SS*)-(**1a**) and (*M-RR*)-(**1b**) (ethanol, 3 °C) show isodichroic points at λ *ca.* 670, *ca.* 540, and *ca.* 377 nm (Figure 2). The same holds true for other solvents and for the pair (*P-SS*)-(**1b**)/(*P-RR*)-(**1a**) (not shown). For species interconvertible by equilibria (*a*) and (*b*) the Δε values belonging to these isodichroic points only differ in sign. Therefore the diastereoisomeric distributions in different media may be determined by c.d. (Table 3). Excellent agreement is obtained with data reported for *rac*-(**1a**)/*rac*-(**1b**) determined by n.m.r. spectroscopy.[9]

If the solvent-dependent population of diastereoisomers exhibiting centrochiral homogeneity (c.e. >95%) is subjected to a closer inspection no correlation with solvent properties is evident. For example, the ratios of diastereoisomers (**1a**):(**1b**) in chloroform and in ethanol are almost identical. On the other hand, dichloromethane and carbon tetrachloride favour different populations of the diastereoisomers. These irregularities suggest that equilibrium positions are not controlled only by differences in solvation energies. However, the isomeric distribution correlates fairly well, with the quotient of dipole strengths $f = D$(u.v.)/D(vis) for (**1**) obtained in different media (Table 2) although corresponding dipole strengths (*D*) of individual isomers are almost identical for the same solvent.* This kind of solvatochromism has long been observed for non-bridged bilatrienes.[7] Thus *f* values are larger for solvents favouring the population of (**1a**) species (*f ca.* 2.7) than for those in which the (**1b**) species predominate (*f* = 2.4–2.5). On the basis of theoretical calculations[14] these variations may be associated with slight changes in helix geometry. Accordingly, *f* increases or decreases if the helix becomes flatter or steeper, respectively. Hence, if this correlation is not accidental, populations of diastereoisomers (**1a**) increase relative to (**1b**) if solvents favour a flatter helix. This interpretation would parallel stereochemical relationships as the methoxy groups are arranged differently.[9]

Conclusions

The success of the unique approach used here for the resolution and isolation of the four stereoisomers of (**1**) mostly rests upon (*i*) the relatively slow interconversion rate[10] of the bilatriene helices, which allows this process to be controlled within a convenient temperature range, and (*ii*) the small energy differences between diastereoisomers (**1a**) and (**1b**) which are of the order of chiral discriminating interactions[13] and which provide significantly different equilibrium positions of the two thermally interconvertible pairs.

The influence of the chirality centres on helicity and h.e. of the bilatriene entity as reflected in equilibria (*a*) and (*b*) should be carefully distinguished from that experienced by covalently bound, optically active peptides or amino acids[4,8,17,18] which may attain numerous spatial orientations as in compound (**2**). In the latter case, close contact of the bilatriene moiety with its peptidic discriminator is possible and is a prerequisite for large h.e.s. Accordingly, increased competition between intramolecular chiral forces and the surrounding solvent diminishes the discriminatory efficiency by increasing the distances between mutually interacting entities. The mechanism of chiral induction operative in bilipeptides might thus be compared with the intermolecular influence of (*R*)-(−)-mandelic acid. Typically,

* This precludes this order arising from spectral differences in (**1a**) and (**1b**) species.

with this kind of discriminator, a change in the preferred helical sense with solvent does not occur. On the other hand, in the bridged, rather rigid, biliverdins (**1**) chiral discrimination is not a consequence of close contact of donor and acceptor sites located at the bilatriene moiety and the chirality centres, but rather arises from slight differences in rotational strain of (**1a**) and (**1b**) type species. Changes in the equilibrium constant K_1 with solvent originate from conformational changes accompanied by solvation stabilising a flatter or steeper helix type. Due to rotational restrictions, spatial relationships between ligands at the chirality centres and the bilatriene moiety can only be changed if the helix inverts. Since non-bonding interactions of (**1a**) and (**1b**) type isomers depend differently on helix geometry and because of the small energy differences involved, solvent-induced variations in excess population may even affect the preferred helicity of the bilatriene backbone. If rotational restrictions are absent as in the centrochiral non-bridged biliverdin given in ref. 5, the diastereoisomers which can be frozen out at −70 °C are present in almost equal populations, as revealed by ^{1}H n.m.r. spectroscopy.

Experimental

The synthesis of compounds (**1**) has been described elsewhere.[9] ^{1}H N.m.r. spectra (250 MHz) were obtained with a Bruker WM 250 spectrometer in [$^2H_6$] benzene with $SiMe_4$ as an internal reference. C.d. and u.v.–vis spectra were recorded with a Jobin Yvon Mark III and a Perkin-Elmer Lambda 7 instrument (equipped with data station 3600), respectively in thermostatted (±0.1 °C) quartz cuvettes of variable path length (0.01–1 cm) in spectroscopic grade (Uvasol, Merck) benzene, chloroform, carbon tetrachloride (all chromatographed on alumina prior to use), dichloromethane, ethanol, and tetrahydrofuran (distilled from $LiAlH_4$ prior to use). Hexamethylphosphoric triamide (HMPA) (Merck) was distilled *in vacuo*. Optical rotations were measured with a Perkin-Elmer 241 polarimeter in thermostatted 10 cm quartz cuvettes. The optically active acids used showed satisfactory optical rotations: (*R*)-(−)-mandelic acid {Fluka, $[\alpha]_D^{20}$ −142.2 ± 0.1° (*c* 5, H_2O)}, (*R*)-(−)-2-phenylbutyric acid {Sigma, $[\alpha]_D^{20}$ −93.2 ± 0.5° (*c* 1, toluene)}, (*S*)-(+)-lactic acid {Fluka, $[\alpha]_D^{20}$ −12.9 ± 0.2° (*c* 2.5, 1.5 mol dm^{-3} NaOH) contained 33% lactide (by titration)}, (*S*)-(−)-2-chloropropionic acid {Sigma, $[\alpha]_D^{25}$ −13.9 ± 0.1° (neat)}, and (*1S*)-(+)-camphorsulphonic acid monohydrate {Merck, $[\alpha]_D^{20}$ +20.5 ± 0.1° (*c* 7, H_2O)}, and were used without further purification.

Resolution and Separation of the Four Stereoisomers of 2,18-(1′,4′-*Dimethoxybutane*-1′,4′-*diyl*)-8,12-*bis*-(2″-methoxycarbonylethyl)-3,7,13,17-*tetramethylbilin*-1,19-(21H, 24H)-*dione* (**1**). *General.*—All solutions containing the respective isomers obtained by procedures described below can be stored at −70 °C for several weeks. C.d. and u.v.–vis spectra were carried out by injecting 5–10 mm^3 of the respective solution into ethanol at 3 °C correcting for slight isomerisation during the recordings. E.e.s were determined by c.d. and/or n.m.r. spectroscopy after the addition of (*R*)-(−)-mandelic acid. Yields were determined by u.v.–vis spectroscopy. For c.d. and u.v.–vis spectra see Tables 2 and 3, and Figure 2. Equilibration of individual isomers in $CDCl_3$ afforded four superimposable ^{1}H n.m.r. spectra identical with that of the unresolved mixture from *rac*-(**1a**) and *rac*-(**1b**).[9]

Isomers (M-SS)-(**1a**) *and* (M-RR)-(**1b**).Compound (**1**) (12 mg, 0.018 mmol) dissolved in CH_2Cl_2 (5 cm^3) was added to a solution of (*R*)-(−)-mandelic acid (8 mg, 0.053 mmol) in methanol (0.5 cm^3) and the solvents were evaporated *in vacuo* at 5 °C. The residue was dissolved in [$^2H_6$] benzene (1.5 cm^3; chromatographed on alumina prior to use) and the solution was allowed to equilibrate at room temperature for 1 h. For the ^{1}H n.m.r. spectrum of this mixture see Figure 1(*b*) and text. This solution was then applied quickly to four t.l.c. plates at 5 °C (20 × 20 cm, Kieselgel 60 precoated plates, 0.25 mm, Merck) and developed in a thermostatted (−15 °C) tray (toluene–ethyl acetate–propan-2-ol 85:10:5 v/v) [R_F(**1a**) 0.12, R_F(**1b**) 0.40]. The two fractions were each triturated with CH_2Cl_2–MeOH (5:1 v/v) at −30 °C, filtered through glass fibre (0.2 μm, Whatman), and concentrated at 10^{-2} Torr (−30 °C) up to *ca.* 2 × 10^{-3} mol dm^{-3} solutions. This procedure was performed four times with the main fractions. For yields and e.e. see Table 1.

Isomer (P-SS)-(**1b**). The solution of (*M-SS*)-(**1a**) obtained after the fourth enrichment cycle containing 1 mg was evaporated to dryness, dissolved in carbon tetrachloride (0.2 cm^3), and allowed to stand at room temperature for 30 min. After t.l.c. at −15 °C as described above and concentration *in vacuo* two solutions (*ca.* 10^{-3} mol dm^{-3}) containing unconverted (*M-SS*)-(**1a**) (0.10 mg, 10%) and the target isomer (*P-SS*)-(**1b**) (0.70 mg, 70%) respectively were obtained.

Isomer (P-RR)-(**1a**). The solution of (*M-RR*)-(**1b**) (0.6 mg) obtained after the fourth enrichment cycle was evaporated to dryness, dissolved in CH_2Cl_2 (0.2 cm^3) and allowed to stand at room temperature for 30 min. After t.l.c. (−15 °C) as described above and concentration *in vacuo*, two solutions (*ca.* 10^{-3} mol dm^{-3}) containing unconverted (*M-RR*)-(**1b**) (0.05 mg, 8%) and the target isomer (*P-RR*)-(**1a**) (0.40 mg, 62%), respectively were obtained.

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References

1 H. Lehner, S. E. Braslavsky, and K. Schaffner, *Liebigs Ann. Chem.*, 1978, 1990.
2 H. Falk, K. Grubmayr, E. Haslinger, T. Schlederer, and K. Thirring, *Monatsh. Chem.*, 1978, **109**, 1451; S. E. Braslavsky, A. R. Holzwarth, and K. Schaffner, *Angew. Chem.*, 1983, **95**, 670; *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 656.
3 M. S. Newman, R. S. Darbek, and L. Tdai, *J. Am. Chem. Soc.*, 1967, **89**, 6191.
4 D. Krois and H. Lehner, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1523.
5 H. Lehner, W. Riemer, and K. Schaffner, *Liebigs Ann. Chem.*, 1979, 1798.
6 G. Blauer and G. Wagnière, *J. Am. Chem. Soc.*, 1975, **97**, 1949; H. Lehner, C. Krauss, and H. Scheer, *Z. Naturforsch., Teil B*, 1981, **36**, 735.
7 S. E. Braslavsky, A. R. Holzwarth, E. Langer, H. Lehner, J. I. Matthews, and K. Schaffner, *Isr. J. Chem.*, 1980, **20**, 196.
8 D. Krois and H. Lehner, *J. Chem. Soc., Perkin Trans. 2*, 1987, 219.
9 D. Krois and H. Lehner, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2179.
10 D. Krois and H. Lehner, *Montash. Chem.*, 1989, **120**, 789.
11 H. Falk and K. Thirring, *Tetrahedron*, 1981, **37**, 761.
12 E. E. Turner and M. M. Harris, *Q. Rev. Chem. Soc.*, 1947, **1**, 299; B. Bosnich, in 'Fundamental Aspects and Recent Developments in Optical Rotatory Dispersion and Circular Dichroism,' eds. F. Ciardelli and P. Salvadori, Heyden, London, 1977, p. 254.
13 S. F. Mason, 'Optical Activity and Chiral Discrimination,' Reidel, Dordrecht, 1979.
14 M. J. Burke, D. C. Pratt, and A. Moscowitz, *Biochemistry*, 1972, **11**, 4025; Q. Chae and P. S. Song, *J. Am. Chem. Soc.*, 1975, **97**, 4176; T. Sugimoto, K. Ishikawa, and H. Suzuki, *J. Phys. Soc. Jpn.*, 1976, **40**, 258; G. Wagnière and G. Blauer, *J. Am. Chem. Soc.*,

1976, **98**, 7806; H. Falk and G. Höllbacher, *Monatsh. Chem.*, 1978, **109**, 1429; H. Scheer, H. Formanek, and S. Schneider, *Photochem. Photobiol.*, 1982, **36**, 259.
15 E. Haidl, D. Krois, and H. Lehner, *Monatsh. Chem.*, 1985, **116**, 119.
16 D. Krois, in preparation.
17 E. Haidl, D. Krois, and H. Lehner, *J. Chem. Soc., Perkin Trans. 2*, 1985, 421; D. Krois and H. Lehner, *Monatsh. Chem.*, 1986, **117**, 1205.
18 H. Falk, G. Kapl, and W. Medinger, *Monatsh. Chem.*, 1985, **116**, 1065; J. Edinger, H. Falk, W. Jungwirth, N. Müller, and U. Zrunek, *Monatsh. Chem.*, 1984, **115**, 1081.

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