236. Enantiomer-Differentiation Induced by an Enantiomeric Excess during Chromatography with Achiral Phases

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It is shown, in the case of the diketone **2**, that chromatography with achiral phases of a non-racemic mixture of enantiomers can furnish fractions which differ in enantiomeric excess. Such chromatography may, therefore, be used to further enrich a sample in one enantiomer. By the same token, chromatography is not a generally safe method for the purification of the product of an enantio-differentiating process, if the enantiomeric excess of a purified portion of that product is taken to be a measure of the efficiency of the process. The described effect represents an enantiomer differentiation induced solely by an alredy existing enantiomeric excess during chromatography. It thus belongs to a class of effects where the relative amounts of two enantiomers induce an observable difference between them. Such effects are called EE effects. The common principle underlying EE effects is explained by a simple symmetry argument. Since EE effects can also occur during reactions with achiral reagents, further transformations of an enantiomer-enriched product may furnish false information on its enantiomeric excess.

1. Introduction. – The efficiency of a process which produces samples containing more of one enantiomer than of the other (asymmetric synthesis or optical resolution), the so-called *e*nantio-*d*ifferentiating[2]) *a*bility (eda) [1][3]), is usually measured by the *e*nantiomeric *e*xcess (ee)[3]) of a sample produced by that process. The frequent practice of equating ee with the *o*ptical *p*urity (op)[3]) is obviously only meaningful if the absence of impurities in the product sample has been established (*cf.* [5]). For this and other reasons, the products of enantio-differentiating processes are often subjected to purification. For this purification to afford a sample containing only the two enantiomers of a desired product, one frequently has to sacrifice some of that product. This is not a problem as long as it can be assumed that the recovered and the sacrificed sample, after the purification, have the same ee, in which case this ee is the ee of the entire product and thus equal to the eda of the examined process. Such an assumption has indeed been made for purifications by ordinary chromatography, which we shall call ap-chromatography[4]): It is said that 'chro-

[1]) From the planned dissertation of *W. L. Tsai*.

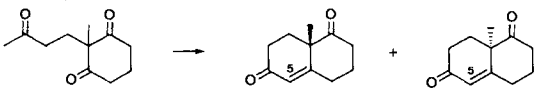
[2]) Meaning according to [1]; a related expression is 'enantioselective' [1], see also [2].

[3]) For the sake of clarity in our discussion, we use certain symbols with the following definitions: F = one enantiomer; F = the other enantiomer. h = amount of F, n = amount of F ($0 < h \geqslant n \geqslant 0$). Enantio-differentiating ability favoring F = eda(F) = $((h-n)/(h+n)) \cdot 100\%$, F and F formed in a given enantio-differentiating process. Enantiomeric excess of F = ee(F) = $((h-n)/(h+n)) \cdot 100\%$, F and F in a sample which may or may not contain impurities; equivalent expressions to ee are 'enantiomeric purity' [3] or 'enantiomeric composition = h/n' [3]. Optical purity of F = op(F) = ($[\alpha]_\lambda^T$ of sample/$[\alpha]_\lambda^T$ of F) $\cdot 100\%$, F in a sample which may or may not contain other impurities than F [4].

[4]) We use the short expression 'ap-chromatography' for achiral-phase chromatography in which both the stationary phase and the mobile phase without the substrate are achiral.

matographic methods can be safely used' [5] to purify the product of an enantio-differentiating process, because 'no enrichments of the enantiomers can occur' [6] by this method, that 'chromatographic methods in an achiral environment' is a 'recommended purification' of a 'sample of unknown enantiomeric purity' [7], and that for 'chromatographic methods', to produce 'differences in retention times or in R_f due to enantiomers, either the stationary phase or the mobile phase must be optically active' [8]. Recently, two papers have appeared in the chromatography literature, one by *Cundy* and *Crooks* [9] on radioactive nicotine and the other by *Charles* and *Gil-Av* [10] on peptides, which suggest that the above-mentioned assumption is not generally valid. We now report on an (independently made) analogous observation with a carbocyclic example [11] well known [12] in enantio-differentiating synthesis, where ap-chromatography[4]) of a sample with a non-racemic mixture of enantiomers has produced an enantiomer enrichment, and discuss a possible cause and some consequences of this effect.

2. Results. – Our observation was made during an investigation of the aldol cyclization[5]) of the saturated triketone **1** catalyzed by a chiral reagent to give samples of the unsaturated diketone **2** consisting of unequal amounts of the (*R*)- and the (*S*)-enantiomer. When the unreacted starting material **1** (42%) was removed by a rough ap-chromatography[4])[6]), the $[\alpha]_D^{25}$ value of **2** varied between the extremes of 54 and 73°. Thus, a sample of **2** with $[\alpha]_D^{25} = +65.0\pm0.5°$ ($c = 0.895$)[7]) was subjected to another ap-chromatography[4])[6]) and collected in four fractions of about equal area under the detector curve: The dried residues of these fractions (64.2, 64.9, 54.3, and 39.7 mg) showed $[\alpha]_D^{25}$ values of +68, +65, +63, and +54° (all ±0.5°, c = between 0.920 and 0.975, measured on aliquots), respectively, corresponding to op = 68, 65, 63, and 54% (*S*). We received the impression that this chromatography had enriched the (*S*)-enantiomer in the first fractions and that later fractions were left with gradually decreasing amounts of the (*S*)-enantiomer.



1 (*S*)-**2** (*R*)-**2**

In order to exclude the possibility that impurities in the product of the reaction **1**→**2** were responsible for the chiroptical differences of these fractions, a pure sample of **2** with ee = 65% (*S*) was prepared by mixing 70 mg of highly purified *rac*-**2** (m.p. 49.0–49.2°, $[\alpha]_D^{25} = 0.0\pm0.5°$ ($c = 0.900$)) with 130 mg of highly purified (*S*)-**2** (m.p. 48.7–49.0°, $[\alpha]_D^{25} = +100.0\pm0.5°$ ($c = 0.920$))[8]). The fact that this sample showed $[\alpha]_D^{25} = +65.1\pm0.5°$ ($c = 1.002$), op = 65% (*S*), can be taken as a first indication of a nearly linear relationship[9]) between op and ee in the case of **2** in *ca.* 1% benzene solution. This sample was ap-chromatographed[4])[6]), and the entire material was collected in ten fractions of about

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[5]) It is the (*S*)-proline-catalyzed reaction first described by *Hajos et al.* and by *Eder et al.* (see [11]), but with a modified catalyst which will be described in another paper.

[6]) Silica gel *(Lobar-A Merck)* with hexane/EtOAc 4:1, 4.0 ml/min; differential refractometer.

[7]) All optical rotations of **2** reported here were measured in benzene solutions; enantiomerically pure (*S*)-**2** shows $[\alpha]_D^{25} = +100°$ ($c = 0.901$, benzene) [13].

[8]) We are grateful to Dr. *A. Kaiser* at *F. Hoffmann-La Roche & Co. AG*, Basel, for a generous gift of *rac*-**2** and (*S*)-**2**.

[9]) That op and ee, in general, need not be in a linear relationship, was shown by *Horeau* [14].

equal area under the detector curve. The exhaustively distilled residues of these fractions (14.7, 19.0, 19.0, 20.2, 20.1, 21.5, 20.7, 18.3, 21.6, and 20.8 mg; 98% recovery) showed $[\alpha]_D^{25}$ values of +84.2, +77.5, +69.6, +66.5, +63.5, +61.5, +59.8, +58.8, +56.4, and +51.3° (all ±0.5°, c = between 0.735 and 1.080), respectively, corresponding to op = 84, 78, 70, 67, 64, 62, 60, 59, 56, and 51% (*S*). Thus, the above-mentioned enrichment of the excess enantiomer by ap-chromatography[4]) was confirmed.

The ee values of the materials **2** in the first and the last of these fractions were determined by ^{1}H-NMR in the presence of an 8- and 3-fold weight, respectively, of $Eu(dcm)_3$ in $CDCl_3$: From the integrations of the differently shifted signals of H–C(5)[10]), the first fraction was estimated to have ee = 84% and the last one ee = 54% (both±2%). The ee values of the second, the fifth and the ninth fraction were also determined by a chiral phase gas chromatographic enantiomer analysis[11]) [15] on a commercially available column[12]); they were found to be 80, 68, and 60% (all ±1%), respectively. The closeness of our op and these ee values confirm the essential linearity of the op to ee relationship[9]) in the case of **2** and further show that no extraneous impurities had been introduced after mixing pure *rac*-**2** and pure (*S*)-**2**.

When exactly the same conditions of ap-chromatography[4])[6]) were applied to 201 mg of pure (*S*)-**2** ($[\alpha]_D^{25}$ = +100.0±0.5° (c = 0.920)) and the eluate devided into four fractions of about equal area under the detector curve, the four exhaustively distilled residues (59.0, 47.1, 44.1, and 49.6 mg; 99% recovery) all had $[\alpha]_D^{25}$ values of +99.5 ± 0.5° (c = between 0.935 and 1.180, measured on aliquots). The same procedure on the same column with 225 mg of *rac*-**2** ($[\alpha]_D^{25}$ = 0.0 ± 0.5° (c = 0.900)) afforded four such fractions, the dried residues of which (49.0, 48.4, 49,6, and 61.6 mg; 93% recovery) all had $[\alpha]_D^{25}$ = 0.0 ± 0.5° (c = between 0.900 and 0.905, measured on aliquots). These two results show that the effect reported here is not due to racemization or some decomposition on the chromatography column nor to undetected impurities either in the samples or on the column.

3. Discussion. – 3.1. *Enantiomer Enrichment.* Our results confirm the suggestion [10] that simple chromatography may be a useful method for the enrichment of one of the enantiomers from a mixture in which that enantiomer is already in excess. By a fractionation type of repetition of this procedure one might, under favorable circumstances, be able to separate the excess enantiomer from the residual racemic mixture.

3.2. *EE Effects.* The chromatographic effect mentioned in *Chap. 2* belongs to a class of effects, where a difference in scalar[13]) properties between two enantiomers F and ⅎ is observed depending on their relative amounts h and n in their mixtures. Since h and n are usually expressed as *e*nantiomeric *e*xcess ($0\% \leqslant ee \leqslant 100\%$)[3]), we shall refer to the class of these effects as *EE effects*[14]). They have previously been observed (but not referred to

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[10]) H–C(5) of (*R*)-**2** was shifted further downfield than H–C(5) of (*S*)-**2** by $Eu(dcm)_3$ in $CDCl_3$; the two signals were baseline separated.

[11]) (*R*)-**2** migrated more rapidly than (*S*)-**2** on the chiral GC column[12]); the two peaks were not quite baseline separated.

[12]) WCOT-fused silica 50 m × 0.22 mm coated with *XE-60-S*-valine-*S*-α-phenylethylamide from *Chrompack*, Netherland.

[13]) A property is understood to be 'scalar', if it is observable by an achiral measurement.

[14]) Some of these effects have been called 'statistically controlled associate diastereomerism (SCAD)' [16], 'self-induced nonequivalence' or 'autononequivalence' [17], 'antipodal interaction' effect [18], 'self-resolution' [19], 'self-association' [10] [20], 'autoassociation stéréosélective' [21], 'chiral discrimination' effect [22].

under this name) in crystallization [5] [22–24], sublimation [25], thermo-diffusion [26], precipitation [27], special extraction [27] [28], polymerization of amino acids [29] [30], hydrolysis of peptides [31], and in a few other reactions [18] as well as in NMR [4] [16] [20] [32] and IR measurements [21] [33] [34]. In some of these papers [10] [30] [31], what we call EE effects have been associated with speculations on the origin of life.

EE effects have in common that they are interpretable by a mere symmetry argument which may be called the EE principle: It considers two enantiomers[15]) in their mixtures not just as isolated molecules F and Ɜ, and thus always as isometries[16]), but together with their surrounding molecules (F_k $Ɜ_l$). These situations F (F_k $Ɜ_l$) and Ɜ (F_k $Ɜ_l$) in non-racemic mixtures (0% < ee < 100%, *i.e.* $k > l > 0$) represent anisometries[16]), and thus must exhibit some scalar property differences so that an EE effect may occur[17]). Only in racemic mixtures of F and Ɜ ($k = l > 0$, *i.e.* ee = 0%), do F (F_l $Ɜ_l$) and Ɜ (F_l $Ɜ_l$) represent isometries[16]), so that F and Ɜ in those mixtures must exhibit the same scalar properties and an EE effect may not occur. An EE effect is, of course, also not observable in the limiting case when Ɜ is absent ($k > l = 0$), *i.e.* in samples with ee = 100%[18]).

As expected from the EE principle, no chromatographic EE effect was found with a racemic mixture (ee = 0%) of **2** in the present work (see *Chap. 2*); the same observation has been made with racemic mixtures in connection with other EE effects [4] [9] [16] [20] [32].

The influence of molecular surroundings on enantiomers causing an EE effect has been explained by thermodynamically non-ideal behavior of molecules, *i.e.* by molecular interactions. In most specific cases, these interactions are thought to be associations [4] [16] [17] [20] [32]. In many cases, such as also in the chromatographic results of *Cundy* and *Crooks* [9] and of *Charles* and *Gil-Av* [10], the associations have been attributed to H-bonding[19]). While intermolecular H-bonding may indeed be a factor for sizeable EE effects because it can cause molecular associations already in relatively dilute solutions, other associations such as due to *van der Waals* forces can also play a role.

3.3. *Molecular Association in* **2**. From the structure of **2** we infer that H-bonding between its molecules can hardly be of importance for our chromatographic EE effect. Furthermore, we found evidence for non-ideal behaviour of (*S*)-**2** in benzene solution only at higher concentrations: The $[\alpha]_D^{25}$ value of pure (*S*)-**2** in that solvent remained constant in the range of $c = 0.080$ to 2.050 (0.005 to 0.12M) at +100.0±0.5°; however, it began to change at $c = 2.980$ (0.17M), increasing from +101.0 ± 0.5° up to +109.0 ± 0.5° at $c = 38.08$ (2.14M).

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[15]) In this connection, two enantiomeric molecules are also said to be in a 'heterochiral' and two homomeric ones in a 'homochiral' [27] [33] [35] [36] relationship. Other related expressions are 'homochirally and heterochirally similar' [21] [37], 'isoconfigurational and heteroconfigurational' [34], 'like and unlike configuration' [28], 'like and opposite configuration' [20] (see also the definition of 'like and unlike' in [38]).

[16]) An 'isometry' or 'anisometry' in chemistry is the situation where the distances between corresponding atoms in two corresponding arrangements of mobile molecular ensembles are all the same or not all the same, respectively (*cf.* [36]).

[17]) An EE effect may also be too small to be observed by a given method.

[18]) The EE principle also applies to any given energy consideration since energy is just one of the scalar properties referred to.

[19]) In [9] this is called 'differential interaction between like and unlike optical isomers' associated as 'homo- and hetero-dimers'; in [10] the corresponding expression is 'self-association of the solutes', in [16] 'homo and cross associates'.

Keeping in mind that enantiomeric crystal packing forces of **2** are stronger than homomeric ones (*rac*-**2** crystallizes from Et_2O/petroleum ether as a racemate [13]), we looked for a similar EE effect in solution: However, the FT-IR spectra of pure *rac*-**2** and pure (*S*)-**2** in 0.06M hexane solutions showed no noticeable difference[20]), whereas IR differences had been noted [13] between crystalline *rac*-**2** and (*S*)-**2**.

The ^{1}H-NMR spectra of *rac*-**2** and (*S*)-**2** also showed no difference, even when measured in rather concentrated (2.1M)[21]) solutions in $CDCl_3$ as well as in C_6D_6. Despite its sizeable chromatographic EE effect, **2** evidently shows no noticeable ^{1}H-NMR-EE effect.

From all this we conclude that associations between molecules of **2** occur at most in relatively dense solutions and that, therefore, any EE effect is likely to become noticeable only at higher concentrations. Since high concentrations cannot be excluded on the surface of the adsorbent, molecular association *may* be responsible for the chromatographic EE effect. In a subsequent paper we shall consider another explanation of the chromatographic EE effect by applying the EE principle to another model.

3.4. *The Need for Precaution in Determining Enantio-Differentiation Abilities.* Since EE effects have been observed in a number of purification procedures (mentioned above), it appears likely that an EE effect cannot, a *priori,* be excluded in *any* particular purification procedure. Special precaution is demanded, therefore, when the eda value[3]) of an enantio-differentiating reaction or similar process, where impurities may occur, is determined by measuring the ee value[3]) of a product sample: *Without* purification, the op value[3]) of that sample should not be equated with its ee value, unless the amounts and the influence of all impurities on the chiroptical properties of the mixture are known, even if there is linear dependence of ee on op. Yet the present work shows that, *with* purification, the ee value of the product sample should not be equated with the eda value of the process, unless it can be demonstrated that any product loss or any EE effect is negligeable in the purification procedure utilized. The best way is to determine the eda value directly on an unpurified product sample, which means that the method used for this must be able to furnish separate information on the amounts of both enantiomers and, at the same time, not be affected by the nature and the amounts of other impurities present. Separation of enantiomers by chiral-phase chromatography (GC [39] or LC [40]) and NMR measurements [41] with chiral-shift reagents may, under favorable circumstances, come closest to fulfilling this requirement.

Since EE effects can also occur in reactions [18], another precaution is needed: When the eda value of an enantio-differentiating process is determined by measuring the ee value of a sample obtained by further chemical transformations, even with achiral reagents, of the product of that process after the enantio-differentiating step, it must be shown that these further transformations proceeded in very high yields or that any EE effect is negligeable in the reactions used.

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[20]) Different IR spectra between racemic and enantiomeric dipeptides in CCl_4 were observed by *Cung et al.* [21], see also [33] [34].

[21]) This concentration should be compared with the smaller concentrations (0.025 to 1.5M) where an EE effect was observed in ^{1}H- and ^{31}P-NMR measurements of other compounds [4] [16] [20] [32].

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