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EPR IN IRRADIATED OBJECTS: ON THE ABSENCE OF REFLECTION IDENTITY OF OPTICAL ANTIPODES[1)]

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Fine structural differences (deviations from mirror equivalency) between optical antipodes are observed by the flash photolysis technique in conjunction with subsequent EPR spectrum identification of the free radicals formed. Compounds of the left (*l*) configurational series are characterized by a closer packing of particles in the crystal lattice than are compounds of the right (d) configurational series.

1. We have previously shown[2,3] that powerful flashes of ultraviolet light (with a light-pulse energy of several hundred Joules) produce irreversible radiation damage in many solid organic substances: free radicals are formed that give intense EPR signals resembling those obtained by action of penetrating radiation on matter.

The following mechanism of free-radical formation has been established (see[3]). Powerful light pulses break R–H bonds in molecules in the surface layers of the irradiated substance, and considerable amounts of "hot" hydrogen atoms H* are formed, with kinetic energies in the range ~1–3 eV. As they diffuse into the interior of the material, the hot H* atoms interact with RH molecules:

$$RH + H^* \rightarrow \dot{R} + H_2 \qquad (1)$$

or (as further study has shown)

$$RH + H^* \rightarrow \dot{R}H_2. \qquad (2)$$

Processes (1) and (2) give rise to accumulation of large amounts (10^{16}–10^{17} ml^{-1} and more) of hydrocarbon radicals in the interior of solid organic materials that have been irradiated with ultraviolet light.[2)]

2. In continuing the study, we have turned our attention to the extremely strong dependence of the radiation yield of free radicals (G_R) on the structure of the crystalline substance being irradiated by ultraviolet pulses. Thus, for example, within a given homologous series of amino acids, the optically-active forms of α-alanine give a large yield of free radicals upon irradiation, but ultraviolet irradiation produces twenty-fold lesser amount of free radicals in racemic (d*l*) α-alanine. Conversely, in the next member of this series, α-aminobutyric acid, G_R is large for the racemate, but $G_R \rightarrow 0$ for the optically-active forms. The next amino acid, norvaline, practically does not react to irradiation ($G_R \rightarrow 0$) in all its forms. However, the optically-active forms of valine (a homolog of norvaline that has the same molecular composition) is among the most easily reactive substances to irradiation (G_R is very large), etc.

Study of the obtained experimental material makes it possible to point out the reason for this thoroughly unusual behavior, which has not been observed in radiolysis experiments. The point is that the process of free-radical formation (1) and (2) undoubtedly depend critically on the energy of the H* atoms: process (1) requires a considerable energy of activation, and hence can occur only for "hot" enough H* atoms; conversely, the probability of "capture" of atomic hydrogen by reaction (2) must increase with decreasing kinetic energy of the H* atoms.

As the H* atoms that arise at the instant of action

1)See [1] for a preliminary communication.

2)Even those substances in which free radicals hardly appear upon prolonged continuous low-power ultraviolet irradiation give a large radiation yield of free radicals when irradiated by powerful ultraviolet pulses. [2,3] Apparently, this indicates that two-photon processes play an important role.

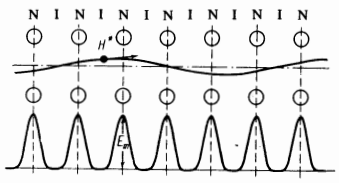


FIG. 1. Sequential passage of an H* atom through potential barriers during diffusion through a "channel" in the crystal structure (diagrammatic). O–atoms of the structure; N–node; I–interstice; E_m–height of the potential barriers. The dot-dash line through the center of the "channel" corresponds to the direction of the crystallographic axis.

of the ultraviolet pulse diffuse into the interior of the material along "channels" of the crystal structure, they successively pass a large number (n) of potential barriers ("apertures" in the interstices); see Fig. 1. If the initial kinetic energy of an H* atom is U_0, and the coefficient of energy loss upon passing a single barrier is α, then in the multiple-passage approximation,

$$U \sim U_0 \alpha^n, \qquad (3)$$

where U is the energy of the H* atom at the end of the diffusion path. Of course, only a small fraction of the H* atoms get into the "channels" of the crystal structure (Fig. 1) and diffuse according to the condition (3). However, only these atoms can be responsible for the formation of free radicals in the interior of the material. The rest of the diffusing H* atoms rapidly lose their energy in chaotic collisions with the crystal structure (their motion is essentially the same in nature as in an amorphous medium).

Let us discuss an example. We shall assume that the difference in values of α for any pair of irradiated homologs is 0.1%. Then, other conditions being the same (including equal initial energies U_0), according to (3), a length of diffusion path of only $\sim 10^{-5}$ cm (10^3 lattice sites) suffices to permit the energies of H* atoms in the crystal specimens being compared to differ by a factor of two. The gross difference that arises in the energies of the diffusing H* atoms is the direct reason for the sharp differences in G_R.

We can easily see that the discussed molecular mechanism indicates that the radiation yields of free radicals in photolysis of crystalline objects are highly sensitive to tiny variations in the coefficient α, or what is the same thing, to tiny regular deviations in the crystal structure.

3. Starting with these ideas, we have tried to apply the method of pulse photolysis with subsequent detection of the produced free radicals with EPR spectra to establish possible differences in the structure of optical antipodes (deviations from mirror equivalence).

Irradiation was performed in air at room temperature. The nominal energy per light pulse (quartz ultraviolet region) varied within the range ~860–330 Joules, depending on the nature of the materials being irradiated. The maximum total radiation dose amounted to $\sim 10^6$ Joules. The design of the illuminator and more detailed information on the irradiation system are given in[2].

Crystalline powders of the optical antipodes were irradiated in pairs. Then they were measured on an

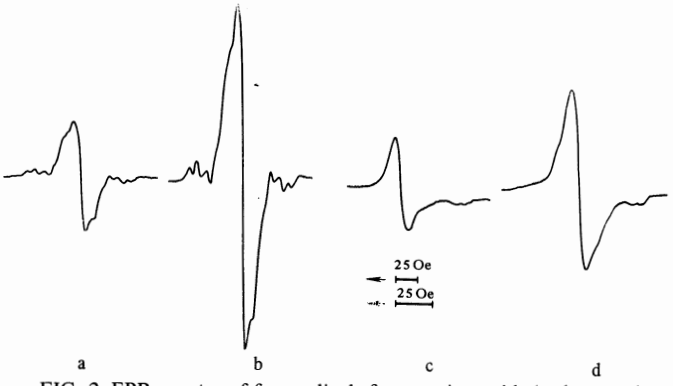


FIG. 2. EPR spectra of free radicals from amino acids (polycrystalline specimens) irradiated by ultraviolet pulses: a) d-β-phenyl-α-alanine; b) l-β-phenyl-α-alanine; c) d-β-phenyl-α-alanine hydrochloride; d) l-β-phenyl-α-alanine hydrochloride. The upper magnetic-field scale refers to spectra a and b, and the lower to c and d. Within each pair, the spectra are on comparable intensity scales. This also holds for the spectra in Figs. 3–6.

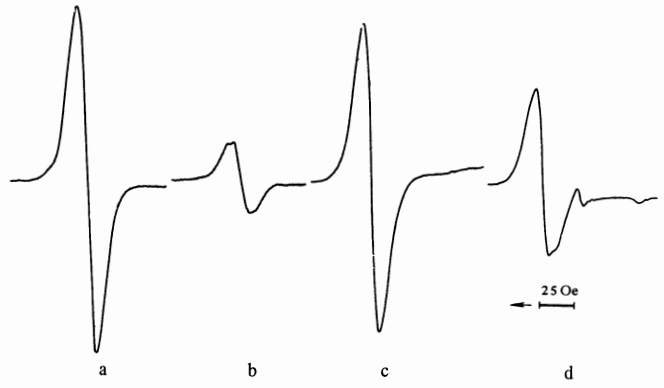


FIG. 3. The same as in Fig. 2, but for: a–d-tyrosine; b–l-tyrosine; c–d-tyrosine hydrochloride; d–l-tyrosine hydrochloride.

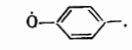
EPR spectrometer (ν_0 = 9320 MHz) under strictly identical conditions, so that the spectra for each pair of isomers (see Figs. 2–6) are comparable in intensity (the accuracy of measurement of intensities was ~10–15%). There is no comparability in intensity of spectra between pairs of isomers.

4. Figures 2a and b show the EPR spectra of crystalline powders of d- and l-β-phenyl-α-alanine irradiated by ultraviolet pulses. The spectra consist of a triplet (1 : 2 : 1), each component of which is split into a quadruplet, and a single line at the center of the triplet. Quite analogous spectra were obtained by radiolysis of frozen benzene and some other aromatic solids. Analysis of the spectra showed that the free radicals contain an extra H atom attached to the benzene ring.[4] This gives grounds for thinking that reaction (2) occurs in the photolysis of phenylalanine, and free radicals are formed by capture of H* atoms by the benzene rings of the molecules of the amino acid. Under the given conditions, the observed manyfold difference in the intensities of the spectra in Figs. 2a and b indicates that the d isomer is more transparent to H* atoms: the value of the coefficient α (Eg. (3)) for the d isomer exceeds that for the l isomer in some place after the decimal point (most probably, no further than the third). That is,

$$\alpha_d > \alpha_l. \quad (4)$$

By recrystallizing the preparations of phenylalanine from aqueous HCl, we obtained well-formed crystals of d- and *l*-phenylalanine hydrochloride. Upon ultraviolet irradiation, their powders gave the spectra shown in Figs. 2c and d. The left-hand intense lines of these spectra ($g_{eff} = 2.00$) belong to phenylalanine radicals, while the weak doublet lines at the right ($g_{eff} \approx 2.02$) belong to $(\dot{H}Cl)^-$ radicals. The line split into a doublet at $g_{eff} > 2$ appears only when one irradiates with ultraviolet those substances whose crystals contain chloride or other halide ions. Apparently, upon irradiation the halide ions capture the H* atoms, and radicals are formed involving the halogen that give a doublet line due to hyperfine interaction with the attached hydrogen atom. The relation of intensities of the spectra in Figs. 2c and d indicates that the condition $\alpha_d > \alpha_l$ holds for phenylalanine hydrochloride as well.

5. The spectra in Figs. 3a and b belong to the d- and *l*-isomers of tyrosine irradiated with ultraviolet pulses. There is every reason to assume that process (1) occurs upon irradiation, and free radicals are formed by detachment of the hydrogen atom from the hydroxyl group of tyrosine. Thus, phenolic-type free radicals are obtained:



However, the unpaired electron is delocalized over the benzene ring. Hence, the line turns out to be greatly broadened by the hyperfine interaction with the protons of the ring, and $g_{eff} = 2.00$. In contrast to the irradiated isomers of phenylalanine (Fig. 2), the d isomer now gives a severalfold larger radiation yield of free radicals. However, since the free radicals of tyrosine were formed by process (1), rather than (2), we conclude as before that $\alpha_d > \alpha_l$.

We see by comparing Figs. 3a and b that the spectra differ not only in intensity but also in form. Most probably, the reason is that in the *l* isomer more radicals (as compared with the total number) are formed near the surface of the material, owing to more difficult diffusion of H* atoms. Upon oxidation by atmospheric oxygen to peroxide radicals,[5] these radicals give an extra line ($g_{eff} > 2$) in the spectrum of the *l* isomer (Fig. 3b). Lines arising from peroxide radicals are not observed when amino acids are irradiated with x-rays, since the radicals are formed uniformly throughout the volume of the material when acted on by penetrating radiation, and the fraction of surface radicals is small.[3)]

Figures 3c and d give the spectra of irradiated d and *l* isomers of tyrosine hydrochloride. The main difference of these spectra from the previous ones (Figs. 3a and b) is that a doublet ($g_{eff} \approx 2.03$; $\Delta H \approx 40$ Oe) arising from $(\dot{H}Cl)^{-1}$ radicals appears in the spectrum of the *l* isomer. The $(HCl)^-$ signal in the spectrum of the d isomer (Fig. 3c) is so weak that it doesn't show up at the chosen amplification of the spectrometer.

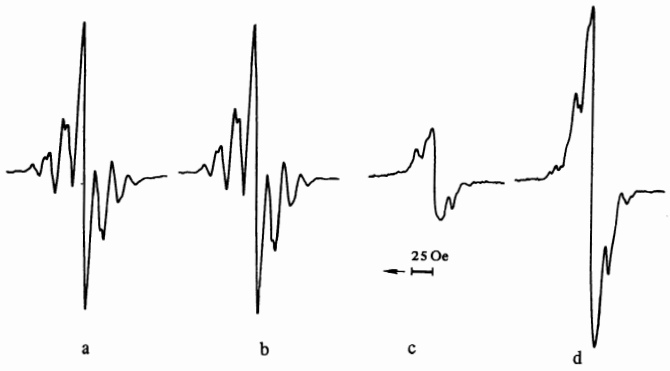


FIG. 4. The same as in Figs. 2 and 3, but for: a–d-valine; b–*l*-valine; c–d-valine hydrochloride; d–*l*-valine hydrochloride (anhydrous).

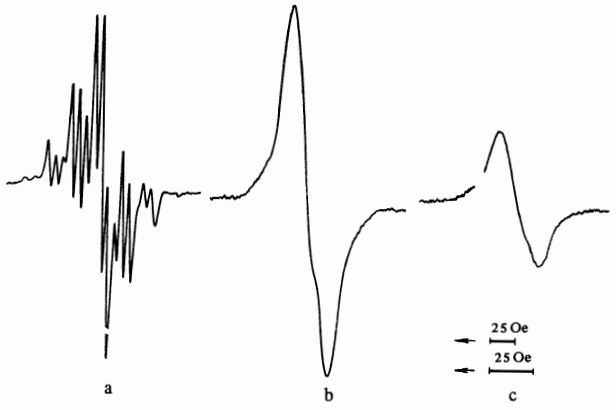


FIG. 5. EPR spectra of free radicals from polycrystalline specimens irradiated with ultraviolet pulses: a–valine prepared by co-crystallization of equal amounts of the dextro and levo isomers; b–d-arabitol; c–*l*-arabitol. The upper scale refers to a, and the lower to b and c.

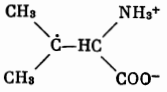
Thus, two types of radicals are formed by ultraviolet irradiation of tyrosine hydrochloride in each of its mirror isomers: phenoxyl, from process (1), and those involving chlorine, from process (2). However, in full accord with the condition $\alpha_d > \alpha_l$, the radiation yield of the former is greater in the dextro isomer (Fig. 3c), and that of the latter in the levo isomer (Fig. 3d).

6. Spectra almost identical in both form and intensity (Figs. 4a and b) are given by crystalline powders of d and *l* valine irradiated by ultraviolet. However, we could unequivocally convince ourselves that the substances being compared are actually carefully-separated optical antipodes by performing a crystallization from an aqueous solution containing equal amounts of each of the isomers. The crystalline powder thus obtained gave an EPR spectrum upon irradiation (Fig. 5a) that fully coincides with the spectrum of the irradiated racemate (d*l*-valine).

On the basis of results of studies on radiolysis of valine[6,7] that gave spectra analogous to ours,[4)] we shall assume that the free radicals

[3)]In addition, there are reasons for thinking that the formation of peroxide radicals of amino acids ($\dot{R} + O_2 \rightarrow R\dot{O}O$) is a specific photooxidation reaction.

[4)]The only difference is that the centers of the spectra in Figs. 4a and b each contain an intense singlet line arising from peroxide radicals. The latter do not arise upon radiolysis (see Sec. 5).

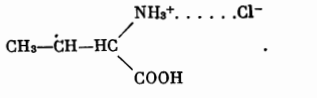


(5)

are formed upon ultraviolet irradiation, and the observed seven fundamental components of the spectra (Fig. 4a and b) result from the equivalent interaction of the unpaired electron with the protons of the two methyl groups. Each of the seven components is split into a triplet by hyperfine interaction with the nitrogen nucleus of the NH_3^+ group.

The radicals (5) could have been formed during photolysis only by detachment of an H atom from a carbon atom of valine, i.e., by process (1). When this fact is taken into account, the identity of spectra in Figs. 4a and b can be simply explained. The optically-active forms of valine are characterized by an extremely high ability to form radicals upon irradiation that is not shared by other aliphatic amino acids: even at ultraviolet doses of only ~150–250 thousand Joules, the concentration of free radicals approaches the maximum possible, as fixed by the conditions of recombination of paramagnetic centers. Apparently, the "apertures" in the interstices of the crystal structure of valine are so large that the H* atoms infiltrate into the interior of the irradiated crystalline particles almost without hindrance (without appreciable energy loss). Naturally, under such conditions the diffusion process described by Eq. (3) proves to be not very critical toward small differences in the structure of the crystals.

However, the picture completely changes after the valine preparations have been recrystallized from aqueous HCl. Figures 4c and d give the spectra of irradiated crystalline powders of the mirror isomers of anhydrous valine hydrochloride. The ability of the preparations to form radicals upon irradiation has been reduced to the usual level, and as we see from these diagrams, the spectra are no longer identical: the *l* isomer is now characterized by a several times larger radiation yield of free radicals than the d isomer. Furthermore, the spectra have become simplified: the number of fundamental components has been reduced to five. On the basis of results of numerous studies on radiolysis (in particular, radiolysis of alanine, polyethylene, etc., see, e.g.[4]), the spectra in Figs. 4c and d can only be ascribed to radicals of the type CH_3-CH-R. In our case, this corresponds to the structure



(6)

(The five fundamental components are due to the equivalent interaction of the unpaired electron with the four protons in the CH_3-$\dot{C}$H- fragment, while the additional splitting of each component into a triplet is due to interaction with the nitrogen nucleus of the NH_3^+ group. In addition, a singlet line arising from peroxide radicals is superposed on the center of the spectrum (see Fig. 4d).

The radicals (6) could not have been formed by process (1), since H* atoms of energies ~1–3 eV are not in a condition to strike off a heavy methyl group

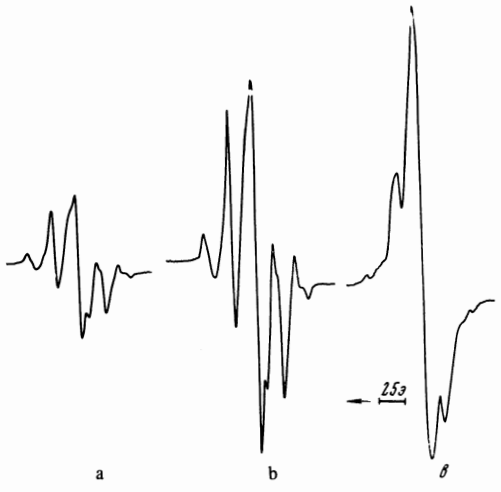
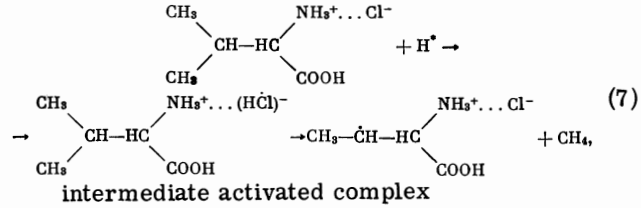


FIG. 6. EPR spectra of free radicals from amino acids (polycrystalline specimens) irradiated with ultraviolet pulses: a) d-α-alanine; b) *l*-α-alanine; c) *l*-glutamic acid.

from the valine molecule. If we take into account the fact that the spectra in Figs. 4c and d lack a doublet line from $(\dot{H}Cl)^-$ radicals, although it is usually observed in the spectra of other optically-active amino acid hydrochlorides (see above), we can represent the process of free-radical formation in the form



(7)

intermediate activated complex

That is, the primary (determining) stage is capture of H* atoms by Cl^- ions (process (2)). On the basis of what has been said, we can rightly consider the several-fold larger radiation yield of free radicals in the *l* form (Fig. 4d) to be another indication of the inequality $\alpha_d > \alpha_l$.

7. In addition to the presented examples, we have observed a manyfold difference in intensity of spectra corresponding to the condition $\alpha_d > \alpha_l$ in the irradiated d and *l* isomers of arabitol[5] (radicals are formed by reaction (1)), as seen in Figs. 5b and c; and in the irradiated d and *l* isomers of α-alanine (the primary radicals being formed by reaction (2)[8]), as in Figs. 6a and b.

8. However, we must note that mirror inequality of optical antipodes is not nearly always observed. We have already discussed above one of the examples (the optical antipodes of valine) (Figs. 4a and b). The reason was the excessive "transparency" of the crystal structure to H* atoms. However, evidently, another limiting case can also occur: conversely, the "transparency" of the crystal structure is too small. This should include substances giving small radiation yields of free radicals.

When the crystal structure is not transparent enough (the coefficient α in Eq. (3) is not close enough

[5]A solid, crystalline alcohol containing five carbon atoms.

to unity), the H* atoms thermalize (come to thermal equilibrium) upon passing a relatively small number of interstices (n). However, $\alpha_d^n \cong \alpha_l^n$ for small n, and the difference between energies of H* atoms in the dextro and levo isomers (U_d and U_l) does not arise.

In full agreement with what has been said, we have not been able to determine a substantial difference in the EPR spectra of even one pair of irradiated optical antipodes that are characterized by a small radiation yield G_R (the intensity of the EPR signals being close to the instrumental noise level prior to synchronous detection). This group of substances includes isohydrobenzoin, serine, norleucine, leucine hydrochloride, and a series of amino acid hydrobromides.

9. All experiments were run in duplicate with preparations obtained from various sources (the Voĭkov Chemical Plant, the firm NBC, "Reanal", "Chemapol", etc.). Supplementary purification by repeated recrystallization was applied. Experiments showed that the presence of microcracks, block boundaries, and other such defects of crystal structure, as well as the degree of comminution of the crystals into powder, had little effect on the results of irradiation. Apparently, as the irradiated surface increases, the probability of exit of unreacted H* atoms from the particles of the material also increases simultaneously. In turn, this rules out an appreciable influence of the cited factors.

10. Thus, it has been shown with the example of seven pairs of optical antipodes that compounds of the levo (*l*) configurational series are characterized by denser packing of the particles in the crystal structure, and hence, they are less "transparent" to H* atoms than the compounds of the dextro (d) configurational series.

Independent confirmation: influence of the observed effect on the melting points of antipodes. Table I, which has been compiled from handbook data,[9-11] gives the melting points (t_m) for the fraction of the substances studied here for which t_m has been measured for both the d and *l* isomers.

Apparently, the non-coincidences of melting points of antipodes (Table I) have been considered somewhat fortuitous (we should take into account that the measurements were performed by different authors at different times). In any case, no fundamental significance was ascribed to them. However, there is a deep correlation between the results of our measurements and the data of Table I, which completely rules out the possibility of an unequivocal interpretation. The point is not only that the greater packing density of the *l* isomers that we have discovered in many cases corresponds to a higher melting point, without one contrary example in which a higher melting point was observed in the d isomer (Table I).[6] The following circumstance is decisive.

As our experiments have shown, optically-active substances for which the t_m of the dextro and levo antipodes coincide (isohydrobenzoin, norleucine, serine, see Table I) give no appreciable radiation yield of free radicals upon ultraviolet irradiation ($G_R \to 0$). Conversely, the substances like valine and glutamic acid that show the maximum difference between the t_m of the antipodes (Table I) are characterized by the highest radiation yield of free radicals upon ultraviolet irradiation. We have already mentioned the anomalously high G_R of the isomers of valine (Sec. 6). Figure 6c shows the EPR spectrum of the free radicals in *l*-glutamic acid[7] (the d isomer of this substance was not available to us). A dose of ultraviolet irradiation of only ~100 thousand Joules sufficed to yield an EPR signal exceeding the instrumental noise level by a factor of many tens. Such a high radiation yield is only slightly less than the radiation yield of valine.

We propose that a unitary explanation can be advanced for the observed regularities. Substances for which $G_R \to 0$ are not very "transparent" to H* atoms. Hence, they are characterized by a denser molecular packing in the crystal structure than the others are. Dense packing (the steric factor) interferes with manifestation of differences between the crystal structures of *l* and d isomers, and consequently, the melting points of the antipodes prove to be equal (isohydrobenzoin, norleucine, and serine; see Table I).

However, the molecular packing should be less dense for substances like valine and glutamic acid whose G_R are anomalously large (the penetrability of the crystal structure to H* atoms is anomalously large). However, the effect of the steric factor declines with decreased packing density, and the structural differences between *l* and d isomers then can be manifested more fully. The latter is expressed in the large melting-point discrepancies of the optical antipodes of valine and glutamic acid (Table 1).

The situation is as if a left-handed torque were acting in the anisotropic (crystalline) medium, leading to compression of crystals made of left-handed molecules (the "left-hand" screw being twisted even more tightly), and rarefaction of crystals made of right-

Table I

Substance	t_m, °C	
	d-isomer	*l*-isomer
α-Alanine	295	297
Valine	293	315
Glutamic acid	213	224—225
Isohydrobenzoin	146	146
Norleucine	301	301
Serine	228	228
Tyrosine	310—314	314—318 (rapid heating)
β-Phenyl-α-alanine	283	283—284

[6] Among 16 other examples of melting points of optical antipodes that could be established from the existing data, [9-11] nine pairs of antipodes had $(t_m)_l > (t_m)_d$ (alloisoleucine, hyoscyamine, threo-isoleucine, proline, 4-hydroxyproline (β-form), tryptophan, cystine, malic acid, and α, β-dibromosuccinic acid). Six pairs had $(t_m)_l = (t_m)_d$ (aspartic acid, borneol acetate, camphoric acid, lysine, 4-hydroxyproline (α-form), and malic acid amide. Only one pair had $(t_m)_d > (t_m)_l$ (the optical antipodes of thyroxine: $(t_m)_d$ = 237°C, $(t_m)_l$ = 235–236°C [9]). We assume that t_m was not measured accurately enough in the latter case.

[7] The spectrum consists of five fundamental components, and most probably corresponds to the free radicals CH_3-$\dot{C}H$-CH(NH_3^+)-COO^-. We can schematically represent their formation by: HOOC-CH_2-CH_2-CH(NH_3^+)-COO^- (a glutamic acid molecule) + H* → HOOC-CH_2-$\dot{C}H$-CH(NH_3^+)-COO^- + 2H → HCOOH + CH_3-CH-CH(NH_3^+)-COO^-.

handed molecules (the "right-hand screw" being partially unscrewed). The steric factor (dense packing) interferes with the action of the stated force.

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