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CHIROPTICAL PROPERTIES OF DANSYL AMINO ACIDS

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Abstract—The ORD curves of several dansyl amino acids have been measured. Most derivatives of the (*S*)-configuration showed a positive Cotton effect at 260 nm. The sign of this effect was rationalized in terms of conformational analysis and was caused by twisting of the sulfonamide group. These results can be employed to determine the configuration of amino acids in natural products.

A number of chromophorically substituted derivatives of the optically transparent amino group have been investigated by means of ORD and CD in order to determine the optical configurations of amino acids.[1]

The most interesting were the derivatives simultaneously used for the determination of N-terminal amino acids residues in peptides and in proteins. Phenylthiohydantoines[2] which are often obtained totally racemized are not useful, more so are methyl thiohydantoines.[3] 2,4-Dinitrophenyl-amino acids[4] and analogous pyridine derivatives: N-(2-pyridyl N-oxide)-amino acids[5] and N-(3-nitro-2-pyridyl)-amino acids[6] were also examined.

The reagent commonly used to determine the sequence of the peptide structure is 1-dimethylaminonaphthalene - 5 - sulfonyl chloride (dansyl chloride).[7] It reacts with peptides and after hydrolysis gives strongly fluorescent 1 - dimethylamino - 5 - naphthalenesulfonyl - amino acids (dansyl amino acids). The latter compounds are resistant to acid hydrolysis and easily detectable with high sensitivity (10^{-11} mole of amino acid).[8] This method is therefore used in the place of others.

We have recently published[9] a method for the preparation of dansyl amino acids and described their physical and spectral data. In this connection we examined their optical properties. Dansyl amino acids gave three bands in the UV spectrum: 334 nm (log ϵ 3·6), 252 nm (log ϵ 4·1), 217 nm (log ϵ 4·5). Maximum at 334 nm probably corresponds to the overlapping bands ^1L_b and ^1L_a of naphthalene, similarly as it does for 1,5-substituted naphthalenes.[10] The ^1B_b band of naphthalene is observed at 217 nm.

The only optically active band is at 252 nm which is probably related to the n-π^* intramolecular charge transfer band (CT)[11] due to a transition accompanied with a partial electron transfer from the electron-donating dimethylamino group to the electron-accepting sulfonamide group through the π system of the naphthalene ring. This is also confirmed by the disappearance of this band in an acidic medium, caused by blockade of the electron pair. The fact that no Cotton effect is detected in the π-π^* transitions at 334 nm can be accounted for by the properties of the transition: the wave function related to ^1L_b and ^1L_a transitions being localized in the naphthalene ring, the rotation strength is almost zero owing to the great distance from the asymmetric centre responsible for the perturbation. In contrast, owing to the strong contribution of the sulfonamide group, the wave function is effectively perturbed by the substituent, and a Cotton effect at 252 nm is observed.

ORD data of dansyl amino acids and their cyclohexylamine salts are summarized in Table 1. ORD measurements are made somewhat difficult by the strong absorption of these compounds.* The ORD curves of dansyl amino acids showed two Cotton effects at 220–350 nm. Of the eleven amino acids investigated, seven dansyl derivatives with the (*S*)-configuration gave positive Cotton effects at 260 nm, and four derivatives (from phenylalanine, β-cyclohexylalanine, methionine and leucine) gave negative Cotton effects. Similar results have been reported for N(3-nitro-2-pyridyl)-,[6] N-(2-pyridyl N-oxide)-[5] and dimedone[12] derivatives of aromatic amino acids, and for methionine.

These earlier results were interpreted as arising from interaction between the substituted chromophore and the chromophore present in the amino acid residue which absorbs in the range

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*Toniolo[13] had stated that "CD investigations of dansyl amino acids did not give encouraging results".

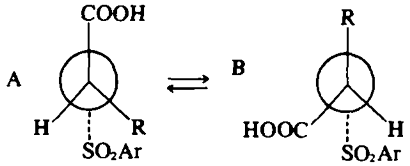
Table 1. ORD data of dansyl (*S*)-amino acids measured in MeOH (molecular rotation values)

Dns-Ala[a,b]	225(0)	229(+8300, pk[c])	241(0)	243(−1700, tr[d])	251(0)	273(+10000, pk)
Dns-Ala		230(+4000, pk)	240(0)	245(−1000, tr)	255(0)	274(+4500, pk)
Dns-Ile[b]	223(0)	225(+9300, pk)	237(0)	243(−3500, tr)	248(0)	272(+12500, pk)
Dns-Ser[b]	225(0)	230(+11000, pk)	240(0)	247(−2000, tr)	255(0)	273(+12700, pk)
Dns-Glu[b]	224(0)	231(+7800, pk)		245(0, tr)		273(+8100, pk)
Dns-Asp[b]		236(+13900, pk)		244(0, tr)		273(+8600, pk)
Dns-Gln		229(+10600, pk)		245(0, tr)		273(+9700, pk)
Dns-Asn		230(+11000, pk)	238(0)	245(−1100, tr)	252(0)	273(+11400, pk)
Dns-Phe[b]			230(0)	251(+10000, pk)	270(0)	275(−2000, tr)
Dns-Phe	220(0)	222(+6000, pk)	228(0)	245(+8000, pk)	257(0)	273(−8300, tr)
Dns-Leu[b]			235(+4000, tr)	255(+11000, pk)	266(0)	280(−3900, tr)
Dns-Leu			236(+4000, tr)	252(+10000, pk)	265(0)	275(−10000, tr)
Dns-Met[b]		224(+14000, pk)	233(0, tr)	252(+2000, pk)	270(0)	282(−1700, tr)
Dns-6HPhe[e]	228(0)	234(−1800, tr)	237(0)	248(+8100, pk)	257(0)	274(−14400, tr)
Dns-βPhe[f]	226(0)	230(+8500, pk)	235(0)	248(−16600, tr)	258(0)	275(+22100, pk)

[a] The symbols are those recommended by IUPAC Commission.[14]
[b] cyclohexylammonium salt.
[c] pk: peak.
[d] tr: trough.
[e] β-cyclohexylalanine.
[f] β-phenyl-β-alanine.

above 215 nm.[5,6,12] However, this hypothesis is not acceptable for the interpretation of our present results. Although the chromophore group in phenylalanine was destroyed, giving β-cyclohexylalanine, the curve of the dansyl derivative of this compound had the same sign as those of dansylphenylalanine and dansylleucine which also has no chromophore absorbing above 215 nm.

We assume on the basis of our results that the change of the curve signs is linked with the change of the conformation equilibrium between Forms A and B:

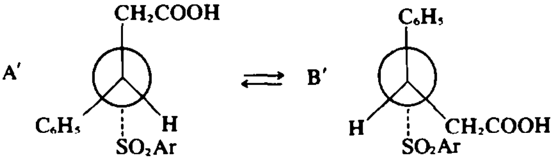


Form A Form B

The Forms A and B correspond to the two staggered low-energy conformers of three possible conformers. This is a consequence of the extremely low steric requirements of the H atom in the asymmetric centre as compared with those of the side chain of amino acid (R) or the carboxyl group.

It can be seen that when the bulkiness of the R group is small (dansyl S-alanine, dansyl S-serine). The sign of the Cotton effect is positive. In this case conformer A is predominant, because groups R and SO_2Ar may still exist in a synclinal system without great steric effect. The contribution of the Cotton effect of conformer B to the total Cotton effect is opposite to that of conformer A. With increasing bulkiness of group R, the population of the B conformers predominates in the whole mixture because groups R and SO_2Ar are converted into the optimal antiperiplanar system.

To the A conformation of dansyl amino acids presumably corresponds the positive Cotton effect, while the negative corresponds to the B conformation. As the conformation equilibrium changes in side B with increase of the bulkiness of the R group, the curve sign also changes. This interpretation is also supported by comparison of the ORD spectra of free dansyl amino acids with spectra of their cyclohexylammonium salts. The amplitude of effect was observed to increase from dansyl alanine to its salt. It decreased in the case of dansyl amino acids which have mainly a B conformation (dansyl S-leucine, dansyl S-phenylalanine). Growing ionization of the carboxyl group changes the conformation equilibrium towards the A form, as a result of increased repulsing between this group and the negatively charged sulfonamide group. The strong positive effect for dansyl S-β-phenyl-β-aminopropionic acid, a member of the opposite configurational series, may be linked with the occurrence of the more stable B′ conformer which is analogous to B.



The sulfonamide group twisted in relation to the naphthalene ring plane under the influence of the hydrogen atom in "peri" position. The main origin of the Cotton effect would be unsymmetrical twisting of the sulfonamide group induced by the neighbouring asymmetrical centre, i.e. the left right-twisted conformers have unequal populations by interaction of the sulfonamide group with the substituents on the asymmetric C atom (Fig 1).

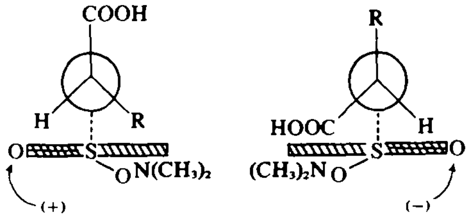


Fig 1. Two predominant conformers of dansyl amino acids. Shaded areas mean the naphthalene ring.

This suggests that clockwise (counterclockwise) twisting of this group corresponds to the positive (negative) Cotton effect when the molecule is viewed from the opposite side to the dimethylamino group in the plane of the naphthalene ring.

The ORD curves of dansyl amino acids (S series) have an additional maximum at 225 nm, which corresponds to the n-π^* transition of the carboxyl group.[15] This feature can be also used to obtain the configuration, but measurements are difficult because of strong absorption in this region.

Our results may serve in studies of the structure of natural products for determining the configuration of dansyl amino acids isolated either from the column or chromatographically.

EXPERIMENTAL

UV spectra were recorded on a Specord model UV-VIS spectrophotometer. ORD measurements have been carried on a Jasco ORD/UV-5 spectropolarimeter at room temp in MeOH, $c = 3.10^{-4}$ mole/l, the cell length was 10 and 2 mm. Dansyl amino acids were prepared from S-amino acids by the method described earlier.[9]

Dansyl S-alanine cyclohexylamine salt, m.p. 152–155° (from MeOH, Et$_2$O); UV: λ_{max} (MeOH) 217, 251, 333 (log ϵ 4·64, 4·16, 3·69 respectively); λ_{max} (0·1N HCl) 223, 288, 321 (log ϵ 4·60, 3·80, 3·45 respectively); (Found: N, 10·13, $C_{21}H_{31}N_3O_4S$ requires: N, 9·98%).

Dansyl S-isoleucine cyclohexylamine salt, m.p. 173–176° (from MeOH, Et$_2$O, petroleum ether); UV: λ_{max} (MeOH) 217, 252, 334 (log ϵ 4·50, 4·13, 3·64 respectively); (Found: N, 9·17, $C_{24}H_{37}N_3O_4S$ requires: N, 9·05%).

Dansyl S-methionine cyclohexylamine salt, m.p. 159–161° (from EtOH, petroleum ether); UV: λ_{max} (MeOH) 218, 252, 334 (log ϵ 4·44, 4·13, 3·66 respectively); (Found: N, 9·03, $C_{23}H_{35}N_3O_4S_2$ requires 8·74%).

Dansyl S-aspartic acid cyclohexylamine salt, m.p. 208–209° (with dec; from EtOH, water); UV: λ_{max} (MeOH) 218, 249, 331 (log ϵ 4·48, 4·15, 3·66 respectively); (Found: N, 9·70, $C_{28}H_{44}N_4O_6S$ requires: N, 9·91%).

Dansyl S-serine cyclohexylamine salt, m.p. 191–193° (with dec; from 90% EtOH); UV: λ_{max} (MeOH) 218, 251, 334 (log ϵ 4·48, 4·15, 3·66 respectively); (Found: N, 9·34, $C_{21}H_{31}N_3O_5S$ requires: N, 9·58%).

Dansyl S-β-cyclohexyl-α-aminopropionic acid, amorphous; UV; λ_{max} (MeOH) 218, 251, 333 (log ϵ 4·50, 4·10, 3·69 respectively); (Found: N, 7·13, $C_{21}H_{28}N_2O_4S$ requires: N, 6·93%).

Dansyl S-β-phenyl-β-aminopropionic acid, amorphous; UV: λ_{max} (MeOH) 217, 252, 334 (log ϵ 4·44, 4·08, 3·64 respectively); (Found: N, 7·14, $C_{21}H_{22}N_2O_4S$ requires: N, 7·04%).

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