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THE AMIDE-AROMATIC-RING SYSTEM

AN INHERENTLY DISSYMMETRIC CHROMOPHORE

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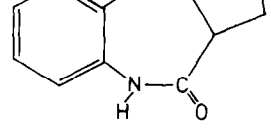
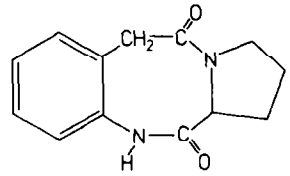
Abstract—Two cyclic peptide like compounds, cyclo-anthranoyl-L- prolyl (**1**) and cyclo-homoanthranoyl-L-prolyl (**2**) have been synthesized and investigated by UV spectroscopy and measurement of circular dichroism. Compound **1** of entirely rigid conformation with two N–CO groups conjugated to the aromatic ring, Ar–NH–CO left handed helical, Ar–CO–N right handed helical, shows a very strong positive Cotton effect centered around 250 nm and a strong negative one centered around 227 nm. Compound **2**, which has only one conjugated skewed electron system, Ar–NH–CO, can exist in two different stable conformations, one of them being left handed, the other one right handed helical. It also shows a strong positive Cotton effect centered around 235 nm. Since from earlier ^{13}C NMR data the latter was concluded to be the preferred conformation, a right handed helical sense of the amide bond-aromatic ring system is likely to correlate with a positive Cotton effect of this inherently dissymmetric chromophore.

Inherently dissymmetric chromophores are light absorbing groups giving rise to extraordinary strong Cotton effects in ORD and CD due to a twisted arrangement of the electron system involved. To compounds of this type belong hexahelicenes, optically active biphenyls, 1,3-dienes and α,β-unsaturated ketones.[1] From our investigations on CD of cyclo-anthranoyl-L-prolyl (c-Ant-Pro, **1**) and cyclo-homoanthranoyl-L-prolyl (c-hAnt-Pro, **2**) follows that in these cyclic compounds the conjugated system formed by the benzene ring and the amide bonds has the properties of an inherently dissymmetric chromophore.



1



2

EXPERIMENTAL

All m.ps are uncorrected.

Spectroscopic measurements. Absorption spectra were recorded on a Unicam AP-800 spectrophotometer, and CD spectra on a Roussel-Jouan Dichrograph II. Molecular weights were determined on a Du Pont 21-492 mass spectrometer by Dr. W. Otting.

Prolyl-anthranilic acid methyl ester. 9.5 g (25 mmoles) of the oily, blocked dipeptide obtained by mixed anhydride condensation of equimolar amounts of N-benzyloxycarbonyl-L-proline and methyl anthranilate, were hydrogenolyzed in MeOH over 10% Pd/C during 2 hr. After removal of the catalyst the filtrate was evaporated *in vacuo*. The product crystallised after trituration with petrolether, yield 2.8 g (45%). Recrystallisation from MeOH gave a substance with m.p. 59–60° and $[\alpha]_D^{20}$ − 7.6 (*c* 1, MeOH). ($C_{13}H_{16}N_2O_3$; mol.wt. 248.3; *m/e*$^+$ 248) (Elemental analysis Calcd: C, 62.88; H, 6.50; N, 11.28; Found: C, 63.42; H, 6.59; N, 11.03%); UV (*c* 5×10^{-5}M, MeOH): ϵ_{305} 5500; ϵ_{259} 11300; ϵ_{252} 14000; CD (*c* 1×10^{-5}M, MeOH): $[\theta]_{305}$ + 3800; $[\theta]_{266}$ − 2700; $[\theta]_{247}$ + 4700; cross-over points 277 and 235 nm (Fig. 3).

Cyclisation failed during heating of the peptide ester in boiling sec BuOH over a period of 5 hr. Only small amounts of cyclisation product were formed after heating without solvent at 110° for 8 hr.

Cyclo-anthranoyl-L-prolyl (**1**). 5.44 g (20 mmoles) of benzyloxycarbonyl anthranilic acid[2] were condensed in chloroform with 3.3 g of the hydrochloride of L-proline methyl ester using equimolar amounts of DCCI and triethylamine. The oily product (6.6 g, 86%) was deblocked by hydrogenolysis over Pd/C in MeOH and the anthranoyl-L-prolylmethylester was heated overnight in boiling MeOH. After removal of the solvent the product was recrystallized by trituration with ethyl ether and filtered. The crystals were washed on the filter with dil HCl and water. Crystallisation from MeOH yielded 1.5 g (40%) with m.p. 209–211°; $[\alpha]_D^{20}$ + 533 (*c* 0.5 MeOH). $C_{12}H_{12}N_2O_2$; mol.wt. 216.2; *m/e*$^+$ 216. (Elemental analysis Calcd: C, 66.65; H 5.60; N, 12.91. Found: C, 66.64; H, 5.88; N, 12.97%); UV (*c* $2.6\cdot10^{-5}$M MeOH): ϵ_{290} 4600; ϵ_{250} 10100; ϵ_{230} 20200; ϵ_{215} 38000. CD (*c* $4\cdot10^{-3}$M MeOH): $[\theta]_{290}$ − 7300; $[\theta]_{248}$ + 196000; $[\theta]_{227}$ − 132000; $[\theta]_{212}$ + 64000; $[\theta]_{200}$ + 6600; cross-over points 217, 236 and 277 nm (Fig. 2).

N-Benzyloxycarbonyl-homoanthranilic acid. 3.8 g (25 mmoles) of *o*-amino-phenylacetic acid[3] was reacted with benzyloxycarbonyl chloride under Schotten–Baumann conditions. After acidification with conc HCl 3.5 g (49%) of crystalline product were obtained, m.p. 111–112° (after recrystallization from MeOH). (Elemental analysis mol.wt. 285.3; Calcd: C, 67.35, H, 5.30; N, 4.91. Found: C, 67.85; H, 5.56; N, 5.28%).

Cyclo-homoanthranoyl-L-prolyl. 3.36 g (11.8 mmoles) of N-benzyloxy-carbonyl homoanthranilic acid were condensed in chloroform with 3.77 g (11.9 mmoles) of the hydrobromide of L-proline *p*-nitrophenylester[4] using equimolar amounts of DCCI and triethylamine. The oily product was deblocked with HBr in glacial AcOH, precipitated with ethyl ether and dried over KOH *in vacuo*. The powder (4.35 g, 77%, no defined m.p.) was heated in 250 ml of spectral grade pyridine over a period of 2 hr. After evaporation *in vacuo* the residue was treated with a little water. The brown, viscous product obtained was thoroughly washed with

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dil HCl and water and thereafter dissolved in MeOH. The soln was decolorized by heating with charcoal and concentrated to a small volume from which a small amount of product (0.3 g) crystallized, m.p. after recrystallisation from MeOH 260–261°; $[\alpha]_D^{20}+737$ (c 0.7 in MeOH). $C_{13}H_{14}N_2O_2$; mol.wt. 230.25; m/e^+ 230. (Elemental analysis: Calcd:C, 67.81; H, 6.13; N, 12.16. Found: C, 67.83; H, 6.42; N, 12.36%); UV (c 8×10^{-5} M, MeOH): ϵ_{270} 2000; ϵ_{232} 11400; ϵ_{214} 18200; CD (c 1.6×10^{-3} M, MeOH): $[\theta]_{230}+205000$; $[\theta]_{215}+119000$; $[\theta]_{200}-84000$; cross-over point 205 nm (Fig. 4).

RESULTS AND DISCUSSION

Cyclo-anthranoyl-L-prolyl (1) has an entirely rigid structure. Both amide bonds are twisted in relation to the plan of the aromatic ring: the group Ar–NH–CO– forming a left-handed helix and the group Ar–CO–N– a right-handed helix (Fig. 1a, b).

Absorption and CD spectra of 1 are shown in Fig. 2. Beside a weak maximum at 290 nm (ϵ 4600) two shoulders, at 250 (ϵ 10100) and 230 nm (ϵ 20200), are observed in the absorption spectrum. With these correspond in the CD spectrum two very strong Cotton effects of an inherently dissymmetric chromophore, a positive one at 250 and a negative one at 227 nm. The absorption band at 290 nm shows only weak optical activity. Two absorption bands at long wavelengths (248 and 290 nm) can be ascribed to a $^1A\rightarrow{}^1L_a$ and $^1A\rightarrow L_b$ transition of benzene.[5]

In derivatives of benzene analogous electronic transitions are taking place but shifted in frequency and intensity. Particularly constant, even in derivatives like 1,2-diamino-4-nitrobenzene, is the $^1A\rightarrow{}^1L_a$ transition[6], in anthranilic acid the characteristic excitations of the benzene ring are shifted to 327 nm (^1L_b) and 248 nm (^1L_a) respectively.[7]

From Fig. 2 it is visible that in 1 from both these excitations only the excitation ^1L_a is revealed by the CD spectrum as the excitation of the inherently dissymmetric chromophore. In the theoretical treatment of UV spectra of aromatic compounds[8] it is assumed that the perturbating substituent would mix various benzene states with each other and with intramolecular charge-transfer states, resulting from the transition of the electron from the substituent to the excited orbital of the ring. This "second order conjugative perturbation" is much larger for $^1A\rightarrow{}^1L_a$ transition than for $^1A\rightarrow{}^1L_b$. This is probably the reason for the differences shown by both aromatic transitions in the CD-spectrum of 1.

The region of 200–240 nm in the CD spectrum of 1 is similar to that of cyclo-tri-prolyl.[9] However, the amplitude of the negative Cotton effect at 227 nm meets the requirements of an effect due to an inherently dissymmetric chromophore. In this region a very weak $n\rightarrow\pi^*$ transition is observed at normal prolyl compounds,[10,11] giving a negative Cotton effect in CD spectrum of polyproline I at about 236 nm[12,13] and of cyclotriprolyl at about 233 nm.[9] Our results indicate that in compound 1 the $n\rightarrow\pi^*$ transition of the amide bond cannot be analyzed isolated from the conjugated aromatic ring. This conjugation with the π bond system of the ring causes the excitation to become that of an inherently dissymmetric chromophore.

Because in 1 both amide bonds conjugated with the

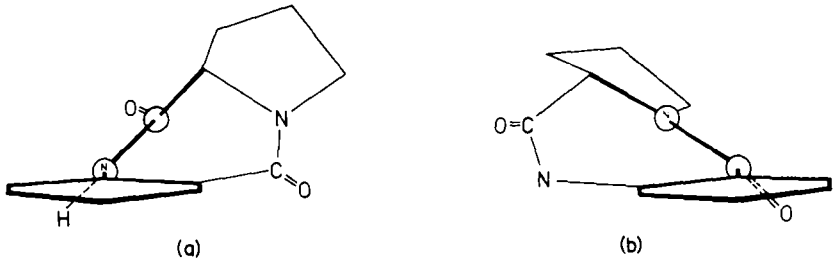

Fig. 1. Cyclo-Anthranoyl-L-prolyl as viewed along Ar–NH–CO– (1a, left handed helix), and along Ar–CO–N– (1b, right handed helix).

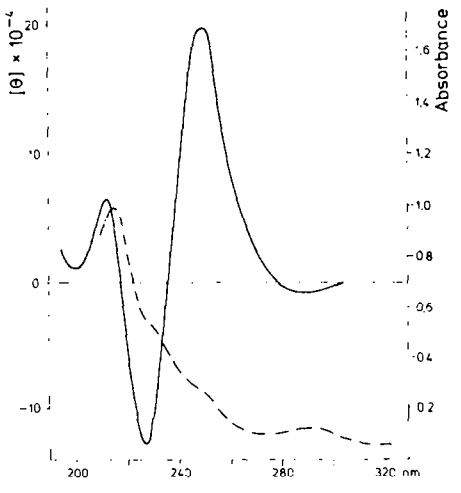

Fig. 2. CD spectrum of c-Ant-L-Pro (1) in methanol (c 4×10^{-3} M, d = 0.01 cm): solid line. UV spectrum of 1 in methanol (c 2.6×10^{-5} M, d = 1.0 cm): broken line.

aromatic ring have an opposite chirality it is impossible to ascribe unequivocally the signs of the Cotton effects to one of the spatial arrangements within the chromophore.

In the absorption spectrum of the linear peptide L-prolyl-anthranilic methyl ester (Fig. 3) three bands (at 305, 259 and 252 nm) are observed, which can be ascribed to the aromatic ring (the band at 259 nm is visible as a shoulder). These bands correspond in the CD spectrum to three only weak Cotton effects. Hence it can be assumed that an aromatic ring in twisted conjugation with an amide bond forms an inherently optically active system.

In compound 2 only one amide bond is conjugated with the aromatic ring. In its UV spectrum (Fig. 4) two aromatic absorption bands are observed, a weak one at 270–275 nm and a relatively strong one (ϵ 11400) at 230–232 nm. The first one (probably a ^1L_b excitation) gives almost no positive Cotton effect, the second, however, causes a very strong one. Thus, similar as in compound 1, the inherent dissymmetry of the chromophore is due to the ^1L_a and not to the ^1L_b excitation. The next positive Cotton effect is marked by a trough in the 215–220 nm region (the range of a $n\rightarrow\pi^*$ amide excitation). Its

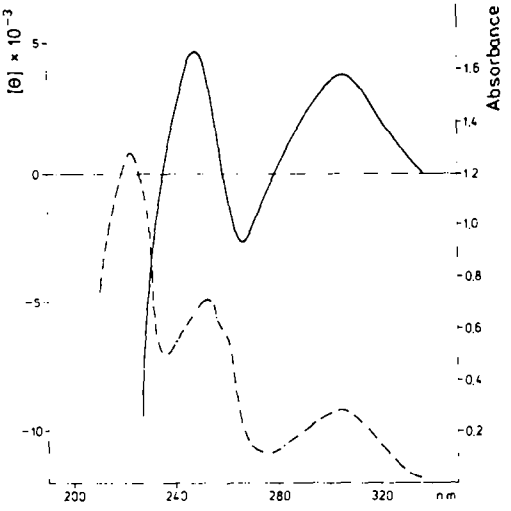

Fig. 3. CD spectrum of H-Pro-Ant-OMe in methanol (c 1×10^{-3} M, $d = 0.1$ cm): solid line. UV spectrum in methanol (c 5×10^{-5} M, $d = 1.0$ cm): broken line.

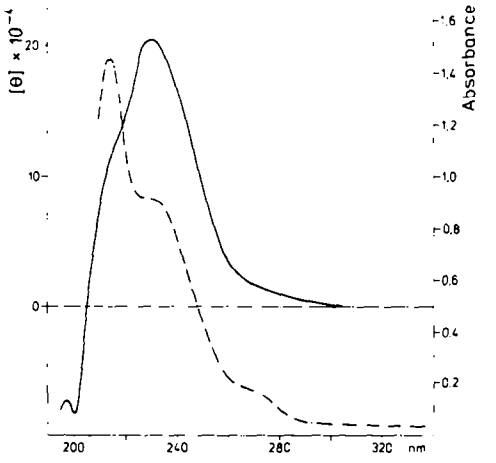

Fig. 4. CD spectrum of *c-h*Ant-Pro (2) in methanol (c 1.6×10^{-3} M, $d = 0.01$ cm): solid line. UV spectrum in methanol (c 8×10^{-5} M, $d = 1.0$ cm): broken line.

intensity can be estimated only with difficulty, because of a partially overlapping of the strong effect appearing at 230 nm.

A Dreiding model of compound 2 indicates that it can exist in two different conformations with a chiral arrangement of the amide bond-aromatic-ring system. In the first one (Fig. 5a) the torsional angle θ in the C^{β}–C^{α}–C′–O group of the proline residue is 30°. Then the central ring of the system exists as a deformed boat and the amide bond and the aromatic ring form a left-handed helix.

In the second possible conformation (Fig. 5b) the value of the torsional angle θ can fluctuate between 70° and 100°, due to a little less stability of the conformation. Here

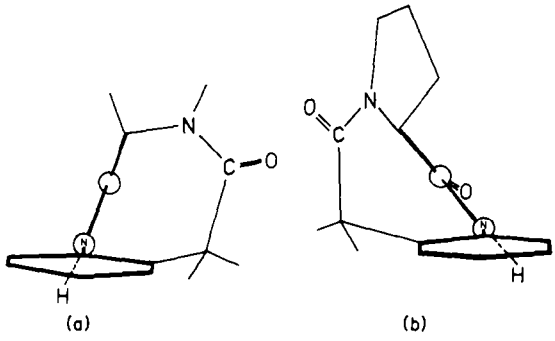

Fig. 5. Two possible conformations of cyclo-homoanthranoyl-L-prolyl (2) as viewed along Ar–NH–CO–. (5a) chromophore forming a left handed helix; (5b) right handed helix.

the amide bond and the aromatic ring form a right handed helix. From our ^{13}C-NMR investigations[14] it appeared that the most probable value of θ in 2 is 75–80°. On this basis the conformation of Fig. 5(b) is more probable than that of Fig. 5(a).

Thus, similarly to twisted 1,3-dienes[15,16] or α,β-unsaturated ketones,[17] and opposite to the twisted biphenyls,[7] a right-handed helical sense of the amide bond-aromatic ring system correlates with a positive Cotton effect of the inherently dissymmetric chromophore.

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