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π...π-INTERACTIONS OF FLAVINS, - II. [1]
[3.3](3,10)ISOALLOXAZINOPHANE AND QUINHYDRONE-LIKE FLAVIN INTERACTIONS

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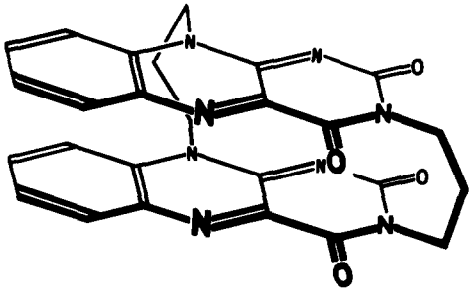
Abstract: To study flavin-flavin interactions [3.3](3,10)isoalloxazinophane was synthesized and its structure determined as 4. Electron spectra including charge-transfer absorptions of semi-reduced states are reported for 4 in comparison with the mono-bridged bis-flavin analogue 3.

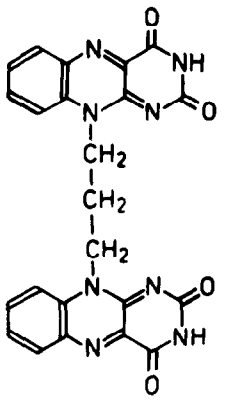
Quinhydrone-like association of oxidized and reduced riboflavins has first been discussed by Kuhn and Ströbele [2], and since then absorption spectra of semi-reduced flavin systems have been measured extensively [3]. The structure of the intermolecular complexes involved, however, is still not well understood. Since flavin-flavin interactions may be relevant for the mechanism of the enzymatic action of complex flavoproteins containing more than one flavin unit [4], more evidence concerning steric and electronic requirements of these interactions was desirable. As in the case of other weak interactions (charge-transfer complexes [5], excimers [6]) the concept of fixing intramolecularly the interacting units in definite orientations has been applied to this problem.



1

In an attempt to synthesize [3.3](3,10)isoalloxazinophane (1), N,N-trimethylenebis(2-nitroaniline) [7] was catalytically hydrogenated and the product was reacted with alloxane monohydrate in analogy to Leonard and Lambert [7]. 10,10'-Trimethylenebis(isoalloxazine) (2) (m. p. 280 - 284°C, dec.) was obtained in 43% yield. For spectroscopic comparison with the doubly bridged [3.3](3,10)-isoalloxazinophane (see below) 2 was methylated (iodomethane, potassium carbonate, dimethylformamide, 36 h, 50°C; 70% yield) to 10,10'-trimethylenebis(3,3'-dimethylisoalloxazine) (3) (small yellow needles from formic acid/water, m. p. 289 - 294°C, dec.). Elemental analysis and spectroscopic data support the

structure of 3 [MS: m/z = 496 (50%, M^+), 382 (23), 270 (48), 268 (85), 253 (21), 242 (22), 228 (85), 183 (29), 182 (28), 171 (32), 170 (16), 143 (100); ^{1}H-NMR (360 MHz, CF_3COOD): δ = 2.87 - 3.01 (m, 2H, CH_2-CH_2-CH_2), 3.76 (s, 6H, N-CH_3), 5.52 ('t', J = 7 Hz, 4H, CH_2-CH_2-CH_2), 8.14 - 8.18 (m centr., 2H, 7,7'-H), 8.41 ('d', J = 8.9 Hz, 2H, 9,9'-H), 8.44 - 8.49 (m centr., 2H, 8,8'-H), 8.55 ('d', J = 8.3 Hz, 2H, 6,6'-H)].



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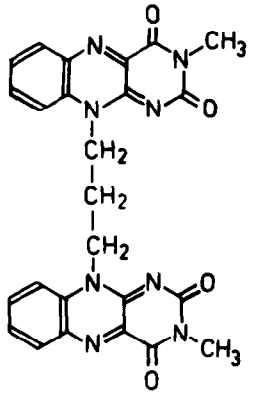
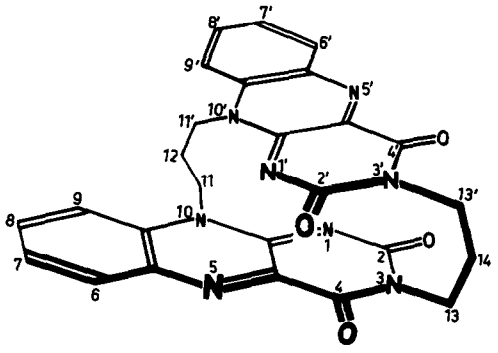


3

To a suspension of 2 and potassium carbonate in dry dimethylformamide within 100 h at 55°C 4.5 equivs. of 1,3-dibromopropane were added. Chromatography on silica (chloroform/formic acid/methanol, 8 : 2 : 0.5) and subsequent crystallization from formic acid/water yielded small yellow needles (10 - 12% yield; m. p. 348 - 350°C, dec.). Structure 1 seemed to be well in agreement with elemental analysis and spectroscopic data [MS: M^+ calc. 508.1607, found 508.1604; m/z = 510 (22%, $(M+2)^+$), 508 (14, M^+), 254 (100), 228 (20), 216 (18), 215 (34), 214 (30), 211 (22), 209 (16), 171 (42), 170 (66), 168 (20), 143 (58)]. A detailed ^{1}H-NMR analysis revealed, however, that the compound formed has not the achiral structure 1 with an ecliptic orientation of the isoalloxazine units but is the chiral isomer 4.



4

In ^{1}H-NMR (360 MHz, CF_3COOD) the aromatic region shows four signal groups for two protons each at δ = 8.12 - 8.16 (m centr.), 8.205 ('d', J ~ 8.6 Hz), 8.37 - 8.41 (m centr.), 8.59 ('d', J ~ 7.6 Hz). Nuclear Overhauser experiments

(NOE) (12-H→ 9-H), see below) in combination with chemical shifts lead to the assignment of these signals to 7,7'-H, 9,9'-H, 8,8'-H and 6,6'-H, respectively. In comparison to 3, of these aromatic protons only the absorption of 9,9'-H is shifted significantly upfield which, as a result of transanular shielding, is more in support of 4 than of 1 where to a certain extent all aromatic protons are expected to be exposed to the deshielding anisotropy effect of the opposite aromatic system. For the six methylene groups of the two bridges six complex multiplets appear between δ = 2.67 and 5.40. By decoupling a correlation of each set of three signals to be assigned to the two different trimethylene bridges was achieved with the result that the multiplets at δ = 3.37 - 3.50, 4.68 - 4.72 and 5.33 - 5.40 belong to the one bridge and the multiplets at 2.67 - 2.80, 4.37 - 4.41 and 4.85 - 4.89 to the other. Since for methyl-substituted isoalloxazines the 10-methyl resonance is generally observed at lower field than the absorption of 3-methyl [8] the first set of signals is assigned to the 10-N...10'-N bridge and, accordingly, the second set to the 3-N...3'-N bridge. This conclusion is supported by the NOE correlation of 9-H (δ = 8.205) to the signal at δ = 3.37 - 3.50 which is the absorption of the two 12-protons in the 10-N...10'-N bridge. 12-H as well as the two protons 14-H in the corresponding central position of the other bridge provide a criterion for distinguishing between the structures 1 and 4. In 1 the protons of each of these two methylene groups are chemically non-equivalent (spin systems of bridge protons: AB-XY-A'B') whereas for 4 they are equivalent by pairs (AB-XX'-A'B'). The observation of only one multiplet each for the two sets of proton pairs favors again structure 4 over structure 1.

The crucial test to prove structure 4 was provided by ^{1}H-NMR measurements in the presence of a chiral solvating agent which with chiral 4, in contrast to achiral 1, was expected to form diastereomeric associates which might differ in ^{1}H-NMR. In fact, in the presence of (+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE, ca. 790 mmol/l) the ^{1}H-NMR spectrum [360 MHz, $CDCl_3$/CF_3COOD (1:1), 303 K] of 4 (ca. 65 mmol/l) showed the signal of the 14-protons, besides strongly highfield-shifted, to be split into two multiplets of the same intensity and shape ($\Delta\delta$ = 0.06); the splitting increased as expected with decreasing temperature ($\Delta\delta$ = 0.083 at 278 K). The solution of 4 (ca. 26 mmol/l) in the presence of racemic TFAE (ca. 1100 mmol/l) under otherwise similar conditions showed for the 14-protons only one single multiplet at δ = 1.98 - 2.08. Obviously, the complexation of the TFAE occurs preferentially at the site of the carbonyl-containing pyrimidine rings of 4. Accordingly, the 3-N...3'-N bridge is more exposed to TFAE than the 10-N...10'-N bridge which within the central part of the molecule is more shielded and whose enantiotopic 12-protons therefore are not split by complexation with (+)-TFAE.

The establishment of structure 4 for the [3.3](3,10)isoalloxazinophane obtained was essential for explaining characteristic differences in the spectroscopic behaviour of quinhydrone-like systems derived from 4 and the mono-

bridged bis-flavin analogue 3. The absorption spectra of 3 and 4 are very similar in showing, with approximately doubled extinction, the typical flavin bands [3: λ_{max} = 434 nm (ε 18550), 338 (15810), 265 (67140); 4: λ_{max} = 430 nm (ε 18240), 341 (17700), 269 (62230), in glycol]. On the other side, in their semi-reduced states 3 and 4 give rise to very different charge-transfer absorptions.

Solutions of 3 and 4 in glycol (c ~ 0.12 mmol/l, 25 ml) were fully reduced by addition of 30 µl of a sodium dithionite solution in water (c ~ 475 mmol/l) under argon; no absorption > 530 nm was observed for the reduced states of 3 and 4 as well as for 3 and 4 themselves. The reoxidation was achieved by carefully shaking the tube with admission of air in small doses until the new absorptions at longer wave-length reached a maximum [3]. In the case of 3 the semi-reduced state obtained in this way showed a strong broad absorption with λ_{max} = 770 nm (ε ~ 680) reaching into the infrared beyond 1100 nm. This absorption is assigned to an intramolecular charge-transfer transition arising from flavin-flavin interaction in a conformation of 3 where the flavin units approach each other in a syn-like conformation allowing favorable overlap between the oxidised and reduced isoalloxazine moieties. The importance of the intramolecular linking is demonstrated by the observation that monomeric 3,6,10-trimethylisoalloxazine under the same conditions does not show a measurable absorption > 530 nm for the formally semi-reduced state.

Surprisingly, the doubly bridged [3.3](3,10)isoalloxazinophane (4) in the semi-reduced state generated in the same way shows a much less pronounced charge-transfer absorption than 3: the broad absorption observed has two not well separated maxima at λ_{max} = 583 and 562 nm (ε ~ 213 and 214, resp.) and does not extend beyond 670 nm (with ε > 50). The difference to 3 is obviously due to the specific orientation of the two flavin units in 4 where overlap between donor and acceptor regions in the semi-reduced state must be rather unfavorable. This result suggests a strong orientation-dependence of quinhydrone-like flavin-flavin interactions which will be further studied on flavinophanes with different orientations.

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