[11] *P. D. Bartlett, S. Bank, R. J. Crawford & G. H. Schmid,* J. Amer. chem. Soc. *87,* 1288 (1965); *P. D. Bartlett & G. Sargent,* ibid. *87,* 1297 (1965); *P. D. Bartlett, W. D. Closson & T. J. Cogdell,* ibid. *87,* 1308 (1965); *P. D. Bartlett, W. S. Trahanovsky, D. A. Bolon & G. H. Schmid,* ibid. *87,* 1314 (1965).
[12] *G. L. Closs, R. A. Moss & S. H. Goh,* J. Amer. chem. Soc. *88,* 364 (1966); *G. L. Closs & S. H. Goh,* J. org. Chemistry *39,* 1717 (1974).
[13] *D. J. Beames & L. N. Mander,* Austral. J. Chemistry *24,* 343 (1971); *D. J. Beames, T. R. Klose & L. N. Mander,* Chem. Commun. *1971,* 773.
[14] *W. F. Erman & L. C. Stone,* J. Amer. chem. Soc. *93,* 2821 (1971).
[15] *M. M. Fawzi & C. D. Gutsche,* J. org. Chemistry *31,* 1390 (1966).
[16] *S. Julia, M. Julia & G. Linstrumelle,* Bull. Soc. chim. France *1964,* 2693.
[17] *F. Arndt & B. Eistert,* Ber. deutsch. chem. Ges. *68,* 200 (1935).
[18] *J. Meinwald & J. K. Crandall,* J. Amer. chem. Soc. *88,* 1292 (1966).
[19] *H. Krieger,* Ann. Acad. Sci. Fennicae Ser. A II. Chim. *109,* 39 (1961).
[20] *J. C. Greever & D. E. Gwynn,* Tetrahedron Letters *1969,* 813.
[21] *J. D. Roberts, E. R. Trumbull, W. Bennet & R. Armstrong,* J. Amer. chem. Soc. *72,* 3116 (1950).
[22] *J. Paasivirta,* Suomen Kemistilehti *36 B,* 156 (1963).
[23] *H. Toivonen,* Suomen Kemistilehti *33 B,* 66 (1960).
[24] *M. Pfau, R. Dulou & M. Vilkas,* C. r. hebd. Séances Acad. Sci. *254,* 1817 (1962).
[25] *R. A. Sneen,* Accounts chem. Res. *6,* 46 (1973).
[26] *G. Fierz & H. Dahn,* résultats non publiés.
[27] *R. W. Taft,* J. Amer. chem. Soc. *74,* 5372 (1952); *F. A. Long, J. G. Pritchard & F. E. Stafford,* ibid. *79,* 2362 (1957); *L. L. Schaleger & F. A. Long,* Adv. physic. org. Chemistry *1,* 1 (1963).
[28] *C. G. Swain & C. B. Scott,* J. Amer. chem. Soc. *75,* 141 (1953); *J. O. Edwards & R. G. Pearson,* ibid. *84,* 16 (1962).
[29] *H. Dahn & J. P. Leresche,* Bull. Soc. vaud. Sci. nat. *70,* 31 (1968).
[30] *C. Wentrup & H. Dahn,* Helv. *53,* 1637 (1970).
[31] *D. M. Brouwer,* Rec. Trav. chim. Pays-Bas *88,* 530 (1969).
[32] *G. A. Olah, Y. Halpern, Y. K. Mo & G. Liang,* J. Amer. chem. Soc. *94,* 3554 (1972).
[33] *G. H. Schmid & A. W. Wolkoff,* J. org. Chemistry *32,* 254 (1967).
[34] *H. Burton & C. K. Ingold,* J. chem. Soc. *1929,* 2022.

272. The Use of Paraquat as an NMR.- and Charge-transfer-Probe for Solvent-Exposed Aromatic Amino-acid Side-Chains[1])

by **Jan W. Verhoeven**[2]), **Anne-Marie A. Verhoeven-Schoff**[2]), **André Masson,** and **Robert Schwyzer**

Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule CH–8049 Zürich

(16. IX. 74)

Summary. The purpose of this investigation was to find new and more potent charge-transfer probes for the study of certain aspects of polypeptide and protein conformation, especially of solvent-exposure of aromatic amino-acid side chains. N,N'-dimethyl-4,4'-dipyridylium ion (paraquat) was shown by NMR. to complex specifically with tryptophan, tyrosine, and phenyl-

[1]) Charge Transfer as a Molecular Probe in Systems of Biological Interest, or Elektronen-Donator-Acceptor-Komplexe bei Polypeptiden, VII. For article VI see [16]. Abbreviations according to the IUPAC-IUB commission on biochemical nomenclature.

[2]) Present address: Laboratory of Organic Chemistry, University of Amsterdam, Nieuwe Achtergracht 129, Amsterdam, The Netherlands.

alanine. The long-wavelength absorption (electronic transitions) typical of charge-transfer complexes was detectable with tryptophan and tyrosine, not with phenylalanine. Paraquat forms slightly stronger complexes than N(1)-methyl-nicotinamidium ion, and appears to have different steric requirements. In human calcitonin and human calcitonin-(11–32)-dokosipeptide all of the aromatic amino-acid side-chains (Phe, Tyr) are accessible to paraquat in solution. This is true also for at least one of the two tyrosines and the tryptophan of ACTH-(1–24)-tetrakosipeptide, and for Trp (62) of chicken egg-white lysozyme. Paraquat is of special interest, because it generates a CT.-absorption band not only with tryptophan, but also with tyrosine, and because of its strong diamagnetic shielding effect.

Introduction. – A variety of spectral probes have been proposed and tested as aids to the study of biological systems on the molecular level [1] [2]. Different molecular environments are reflected by changes of intensity, position, and shape of existing absorption or emission bands.

One of us (*R.S.*) had proposed the use of electron-acceptor molecules as charge-transfer (CT.) probes for amino-acid side-chains with electron-donor properties [3]. CT. probes are rather exceptional in the sense that their 'reporter signal' (*i.e.* the CT. absorption) is a unique property of the complex between the probe and the molecule reported, being completely absent for either of these.

Among the various CT.-probes investigated, electronegatively substituted pyridinium ions seemed most promising due to their stability and solubility in aqueous media [4]. The pyridinium ions used were of the N-alkyl-(iso)nicotinamidium type. These show rather weak acceptor properties, limiting their reporter capability to the strong electron-donor side-chain of tryptophan.

The purpose of the present investigation was to widen the scope of CT. probes for the detection of further aromatic amino-acid side-chains. It was found that paraquat, N,N'-dimethyl-4,4'-dipyridylium dication, is an especially useful reagent. It generates CT.-absorption not only with tryptophan, but also with tyrosine side-chains. Its complexes with these amino-acids as well as with phenylalanine can be detected

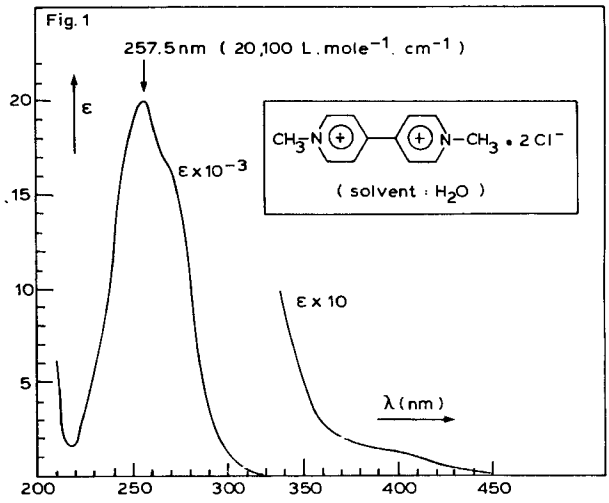


Fig. 1. *Absorption spectrum of paraquat dichloride in aqueous solution at 20°*

by mutual diamagnetic shielding (NMR.) [2] [5]. However, the NMR. technique is presently limited to oligopeptides with relatively simple spectra (*p.e.* ACTH, calcitonin); it has not yet been applied to proteins such as lysozyme, because of spectral complexity (*vide infra*).

Results and Discussion. – The electron-acceptor properties of pyridinium ions can be increased by introduction of electronegative substituents, especially in the 4-position [6]. Such ions are however labile to nucleophilic attack (*e.g.* by OH^-) at higher pH values. Thus the N-methyl-3,4,5-tricyanopyridinium ion is stable only in strongly acidic media [7].

It was found that the N,N'-dimethyl-4,4'-dipyridylium dication (paraquat) [8] combines very good electron-acceptor properties [9] with stability over a large pH

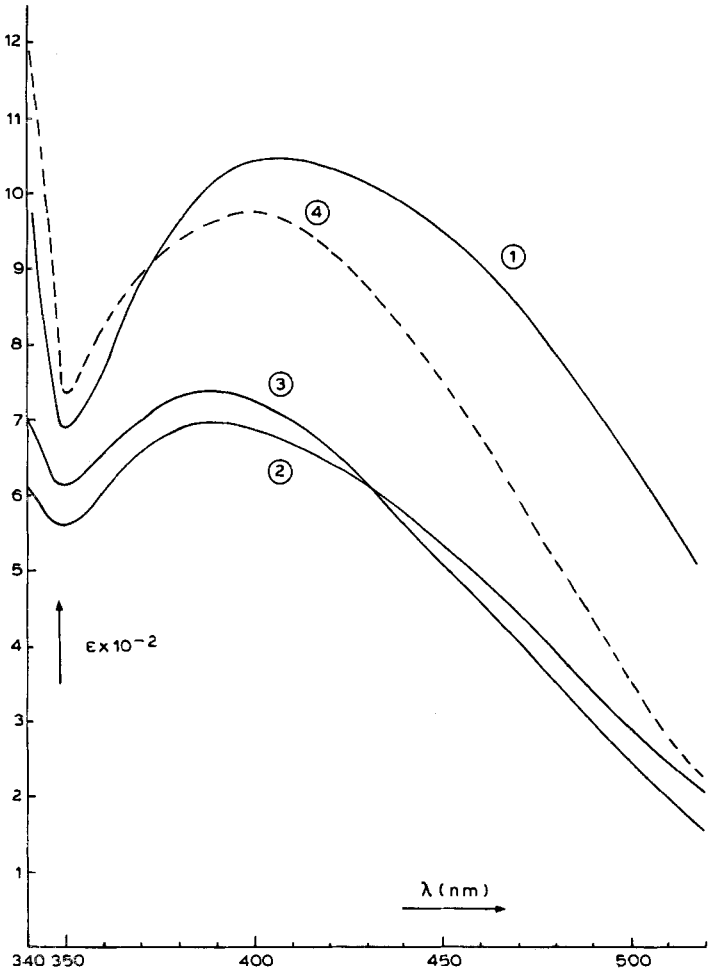


Fig. 2. *Absorption spectra of the complexes between paraquat and skatole (1), N-acetyl-L-tryptophanamide (2), L-tryptophanmethylester hydrochloride (3), and lysozyme (4) in aqueous solution at 20°*

range. Paraquat dichloride forms colourless aqueous solutions which are stable up to about pH 9. The absorption spectrum of neutral solutions is shown in Fig. 1. The very weak long-wavelength absorption observed in concentrated solutions probably stems from CT. interaction with the chloride ion.

A comparison of the CT. absorption maxima observed for complexes of various pyridinium ions with N-acetyl-L-tryptophanamide (Table 1) clearly shows the superior electron-acceptor properties of paraquat. Addition of paraquat to solutions of tryptophan derivatives leads to the appearance of an orange-red colour; a yellow colour is observed with tyrosine derivatives while histidine derivatives lead to a faint yellow at pH > 7 but no coloration below pH $= 6$, indicating a loss of electron-donor properties for the protonated imidazole system. Phenylalanine derivatives do not produce absorption in the visible region with paraquat, although complexation could be shown to occur by NMR. spectroscopy.

Table 1. *Charge transfer absorption maxima resulting from interaction of N-acetyl-L-tryptophanamide with various pyridinium ion chlorides in aqueous solution*

Pyridinium ion	λ_{max} (nm)	Ref.
N-methylnicotinamidium	314	[4]
N-methylisonicotinamidium	355	[4]
N-methyl-4-cyano	378 ± 1	this work
Paraquat	388 ± 1	this work

Spectrophotometric studies on the complexation (binding) of paraquat with tryptophan and tyrosine derivatives. The binding of paraquat to some Trp and Tyr derivatives

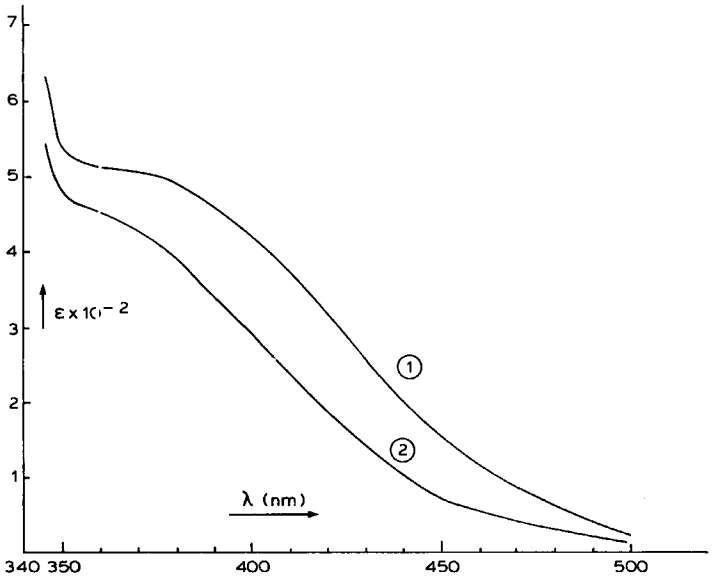


Fig. 3. *Absorption spectra of paraquat/*p*-cresol (1) and paraquat/N-acetyl-L-tyrosineamide (2) complexes in aqueous solution at 20°*

Table 2. *Spectrophotometrically determined parameters for complexes of paraquat with amino acid derivatives and various polypeptides in aqueous solution at 20°.* $\bar{K}$ is the average of the association constants determined at various wavelengths; $\bar{r}$ the average correlation coefficient of the *Scatchard* plots made at these wavelengths

Substrate	370 nm	390 nm	400 nm	410 nm	420 nm	450 nm	$\bar{K}$	λ_{max} (nm)	ε_{max}	$\bar{r}$
Skatole (3-methylindole)	7.20	6.74	–	7.03	6.93	7.12	7.0 ± 0.2	403 ± 1	1050 ± 30	0.988
Ac · Trp · NH_2	5.26	5.71	–	5.55	5.95	–	5.6 ± 0.3	388 ± 1	695 ± 35	0.991
HCl, Trp · OMe	2.32	–	2.88	–	2.64	2.86	2.7 ± 0.2	383 ± 2	740 ± 50	0.972
p-Cresol	2.29	2.12	–	2.08	2.26	–	2.2 ± 0.1	[a]	[a]	0.960
Ac · Tyr · NH_2	1.27	1.67	–	1.56	1.89	–	1.45 ± 0.3	[a]	[a]	0.958
Calcitonine-(11–32)--dokosipeptide [b]	0.6 [c]	–	–	–	–	–	0.6 [c]	[a]	[a]	0.85
Lysozyme	2.46	2.73	2.82	2.80	2.94	–	2.7 ± 0.2 [d]	398 ± 1	980 ± 70	0.968
ACTH-(1–24)-tetrakosipeptide [e]	1.55	1.93	1.93	2.21	–	–	1.8 ± 0.3	370 ± 2	1560 ± 250	–

[a] No discrete maximum observed. [b] In 0.01M acetate buffer; pH = 4.5. [c] Concerns complexation with Tyr-12 (see text). [d] Concerns complexation with Trp-62 (see text). [e] Data from [13].

was studied spectrophotometrically by monitoring the intensity of the CT. absorption as a function of the paraquat concentration. The results obtained for the association constant K, the molar extinction (ε_{max}) of the CT. transition and its position (λ_{max}) are compiled in Table 2. The absorption spectra of the CT. complexes are shown in Fig. 2–3. These spectra were obtained from those observed for the most concentrated solutions in the binding experiments by subtraction of the long-wavelength paraquat absorption and by normalizing to the calculated ε_{max} values.

Comparison of the present data with those reported by *Deranleau* & *Schwyzer* [4a] for systems containing N-methyl-(iso)nicotinamidium ions and Trp derivatives shows that paraquat forms slightly stronger complexes displaying CT. absorption at considerably longer wavelength. Values of ε_{max} are in the same order of magnitude as those for the N-methyl-iso-nicotinamidium complexes. In accord with the results of *Deranleau* & *Schwyzer* [4a] ε_{max} is smaller for N-acetyl-L-tryptophanamide than for skatole. The polar groups in the former probably influence the geometry of the complex and thereby the ε_{max} value. In contrast, however, we observe a rather large decrease of K on going from skatole to N-acetyl-L-tryptophanamide (as well as from

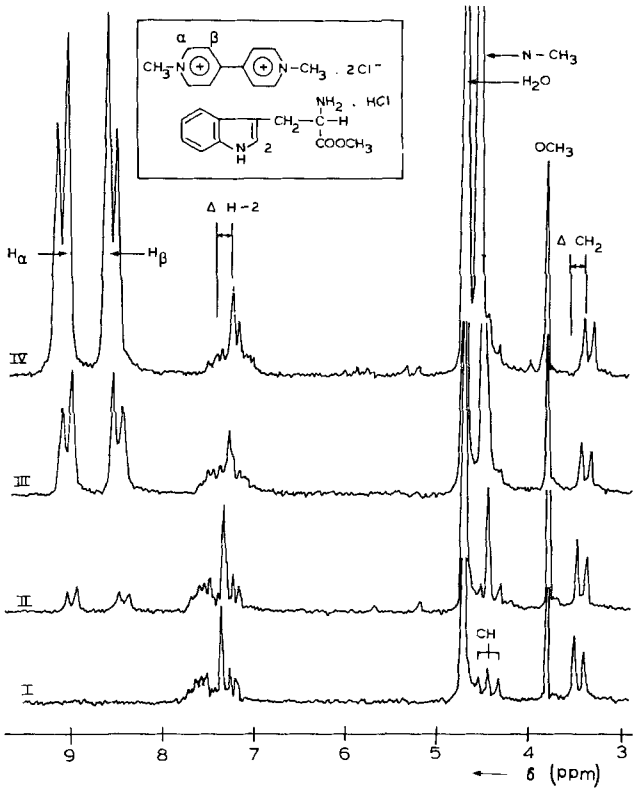


Fig. 4. *60 MHz ^{1}H-NMR. spectra of* L-*tryptophanmethylester hydrochloride (0.1* M *in* D_2O*) containing increasing amounts of paraquat.* (I = none, II = 0.025, III = 0.18 and IV = 0.5 M. The spectra show shielding of TrpOMe, HCl by paraquat (at high paraquat conc.) and of paraquat by TrpOMe, HCl (at low paraquat conc.)

p-cresol to N-acetyl-L-tryosineamide). This might point to a larger sensitivity of the doubly-charged paraquat for environmental factors, as is also shown by the small K found for the paraquat/Trp · OCH_3, HCl complex, where presence of the NH_3^+ group apparently inhibits the efficient complexation of paraquat with the indole nucleus.

^{1}H-NMR. studies on the interaction of paraquat with aromatic amino-acid derivatives. For some aromatic amino-acid derivatives the influence of a large excess of paraquat (0.5 M) on their ^{1}H-NMR. spectra was investigated. The NMR. signals of paraquat do not interfere with those of the amino-acid derivatives studied, as is exemplified in Fig. 4 for Trp · OCH_3, HCl. For all Phe, Tyr and Trp derivatives studied, upfield shifts of protons attached to the aromatic nucleus and to the methylene carbon atom

Table 3. *Upfield NMR. shifts caused by complexation of paraquat with various amino acid derivatives and polypeptides in deuteriumoxide solution at 28°.* Δ = shift observed at a paraquat concentration of 0.5 M. Δ_0 = shift calculated for complete complexation

Substrate	Conc. M	Freq.[a] (MHz)	Signal	Δ (ppm)	Δ_0 (ppm)
HCl, Phe · OMe	0.1	60	Phe-	0.06	[b]
			$-CH_2-$	0.03	[b]
p-Cresol	0.1	60	H-1	0.30	0.57
			H-2	0.37	0.71
			$-CH_2-$	0.20	0.38
Ac · Tyr · OMe	0.002	100	H-1	0.11	0.26[c]
			H-2	0.22	0.52[c]
			$-CH_2-$[d]	0.10	0.24[c]
HCl, Trp · OMe	0.1	60	H-2	0.14	0.25
			$-CH_2-$	0.16	0.28
Ac · Trp · NH_2	0.003	100	H-2	0.26	0.35
			$-CH_2-$[d]	0.22	0.30
Human Calcitonine-(11–32)--dokosipeptide (hydrochloride form)	0.01	100	Phe-16, 19, 22	0.09	[b]
			H-1 } Tyr-12	0.10	[b]
			H-2 } Tyr-12	0.19	[b]
Human Calcitonine (hydrochloride form)	0.01	220	Phe-16, 19, 22	0.08	[b]
			H-1 } Tyr-12	0.08	[b]
			H-2 } Tyr-12	0.16	[b]
ACTH-(1–24)-tetrakosi-peptide	0.002	100	Phe-7	0.14	[b]
			H-1 } Tyr-2,23	0.08	[b]
			H-2 } Tyr-2,23	0.18	[b]
			H-2 Trp-9	0.14[c]	[b]

[a] The following internal references were used: *t*BuOH (1.23 ppm rel. to DSS) in 60 MHz spectra, DSS in the 100 MHz spectra and the methionine-(8) S–CH_3 signal (2.02 ppm rel. to DSS) in the 220 MHz studies on calcitonine.

[b] Association constant not determined or uncertain (Table 2).

[c] Calculated with K determined for N-acetyl-L-tyrosineamide (Table 2).

[d] Forms the AB part of an ABX system. Shifts indicated are the average of Δ_A and Δ_B.

[e] Signal assignment tentative (Fig. 6).

were observed, Table 3. For protons further removed from the aromatic nucleus no significant shifts were found.

These observations point to a specific complexation of paraquat with the aromatic side-chains. In these complexes mutual shielding of the aromatic systems occurs leading to the observation of upfield shifts for all aromatic protons [2] [5]. The observed shifts (Δ) represent a weighted average of the complexed and uncomplexed species. From the association constants determined spectrophotometrically, the shifts for the fully complexed form (Δ_0) were calculated by [5]:

$$\Delta_0 = \Delta(1 + \bar{K}X_0)/\bar{K}X_0$$

where X_0 represents the paraquat concentration. This calculation is rather crude since NMR. and spectrophotometric measurements were made at somewhat different temperatures (28° and 20° resp.).

The values thus obtained for Δ_0 lie in the normal range [5] for face to face aromatic $\pi - \pi$ complexes.

The results of Table 3 suggest that complexation of paraquat with Phe, Tyr and Trp can readily be detected by NMR. spectroscopy for peptides showing sufficiently resolved NMR. spectra. Given a sufficient signal separation, complexation with Phe, Tyr and Trp side-chains could even be detected simultaneously. Spectrophotometrically such a simultaneous observation seems thwarted by the strong overlap of CT. absorption bands resulting from paraquat/Tyr and paraquat/Trp interaction and the absence of a detectable CT. absorption for the Phe/paraquat complex.

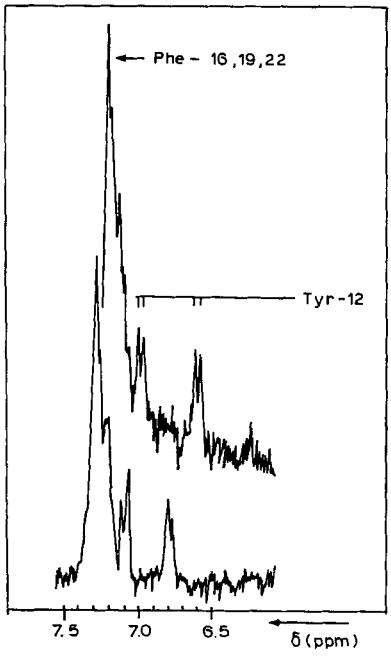


Fig. 5. *Aromatic proton region of human calcitonine, 0.01 M in D_2O, 220 MHz NMR., before (lower trace) and after (upper trace) the addition of paraquat, 0.5 M*

Interaction of paraquat with human calcitonine and human calcitonine-(11–32)-dokosipeptide (part of the dissertation of *A. Masson* [10]). Human calcitonine contains 32 amino acid residues, of which five have an aromatic side-chain: Tyr 12, Phe 16, 19 and 22 and His 20; Trp is absent.

Calcitonine-(11–32)-dokosipeptide was titrated with paraquat at pH 4.5 (0.01 M acetate buffer). A faint yellow colour developed which must be due to Tyr 12/paraquat interaction since His 20 is protonated at this pH [10]. We found $K = 0.6 \pm 0.3$ $(1 \cdot mol^{-1})$ and $\varepsilon = 850 \pm 250$ (at 370 nm), Table 2. A study of the calcitonine-(11–32) NMR. spectrum at 100 MHz revealed upfield shifts for the aromatic protons of Tyr 12 and of all three Phe residues (Tables 3) upon addition of paraquat. Complete human calcitonine also develops a yellow colour with paraquat, and similar upfield shifts of the aromatic protons were observed in the 220 MHz NMR. spectrum, Fig. 5.

The accessibility of 4 amino acid residues along the polypeptide chain as shown by these experiments favours the idea that calcitonine adopts a random coil conformation in solution [10].

Interaction of paraquat with ACTH-(1–24)-tetrakosipeptide. ACTH-(1–24) [11] elicits a biological activity comparable to that of the complete ACTH molecule with 39 amino-acid residues (adrenocorticotropic hormone). It contains 5 aromatic amino-acid side-chains: Phe 7, Tyr 2 and 23, His 6, and Trp 9.

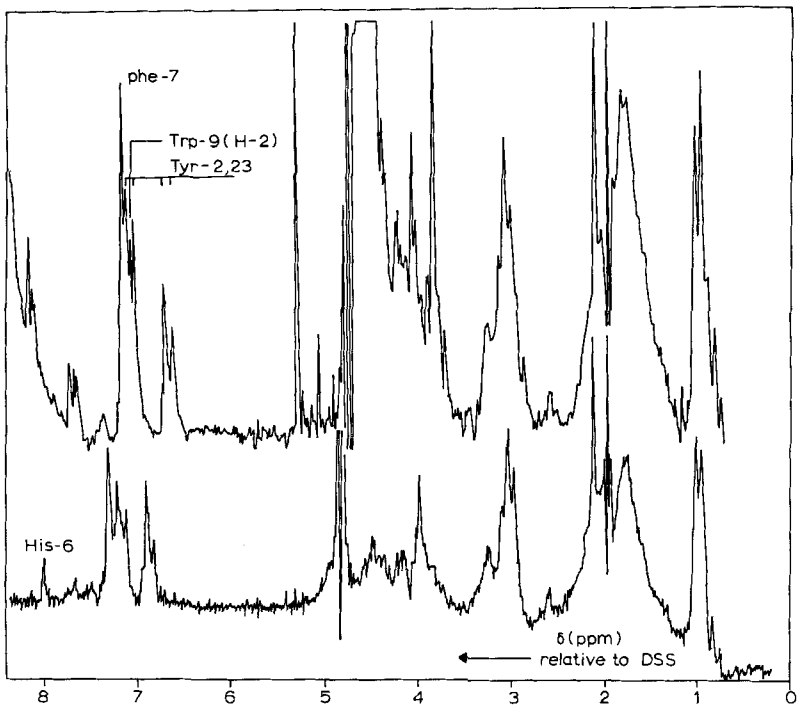


Fig. 6. *^{1}H-NMR. spectrum of ACTH-(1–24)-tetrakosipeptide, 0.01 M in D_2O, 100 MHz, before (lower trace) and after (upper trace) the addition of paraquat, 0.5 M.* The assignment of the H-2 (Trp 9) signal should be considered as tentative

Titrations with N-methyl-isonicotinamidium chloride had already shown [12] that Trp 9 is available for CT. complexation, this was recently confirmed [13] by spectrophotometric titration with paraquat. From these spectrophotometric measurements no direct information regarding the accessibility of the other aromatic amino acids could be gained.

The wavelength dependence of K, the hypsochromic shift as compared to other complexes (Table 2) and the high apparent ε_{max} value observed for the ACTH-(1–24)/paraquat complex appear to provide indirect evidence for the simultaneous complexation of paraquat with at least one Tyr residue besides Trp. The NMR. data of Table 3, however, present direct proof that all aromatic amino acids are accessible for paraquat.

Further studies in stronger magnetic fields to diminish the overlap of the aromatic signals (Fig. 6) and with varying concentrations of paraquat to determine the binding constants for the aromatic amino-acids separately will be needed to clarify the extent of interaction in more detail.

Paraquat and Lysozyme. Chicken egg-white lysozyme forms an orange-red complex with paraquat in aqueous solution. From model studies and from spectrophotometric titrations with N-methylnicotinamidium chloride as an acceptor, *Deranleau et al.* [14] concluded that of the six Trp residues present in lysozyme only Trp 62 is available for complexation. X-Ray analysis (*D. C. Phillips*, personal communication) of a solid lysozyme/N-methylnicotinamidium complex revealed additional complexation with Phe 3.

In principle, a study of the lysozyme/paraquat complex by NMR. spectroscopy could settle the question about the number and nature of aromatic amino-acid side-chains available for complexation. For the time being, however, the rather unresolved NMR. pattern [15] of a large protein like lysozyme impedes such a study.

We have therefore confined ourselves to a spectrophotometric study of the lysozyme/paraquat complex. Excellent linear *Scatchard* plots were obtained in the wavelength region of 370–420 nm; furthermore, no important wavelength dependence of the apparent K values was observed (Table 2). These results indicate that only one (or only one set of equivalent) site(s) for paraquat complexation leading to absorption in this region can be available. This excludes complexation with Tyr residues, but not that with Phe residues. Recent experiments [16] with a 'CT. acceptor-labelled' lysozyme inhibitor leave little doubt that Trp 62 is indeed the site detected spectrophotometrically. The association constant for the Trp 62/paraquat complex is definitely (about 50%) lower than for the simple Trp derivatives studied. With N-methylnicotinamidium chloride as an acceptor such a large difference was not observed [4] [14], which probably reflects the larger steric requirements of paraquat. Furthermore it is found that the Trp 62/paraquat complex exhibits CT. absorption at a remarkably long wavelength. Both λ_{max} and ε_{max} are comparable with the skatole/paraquat complex (Table 2, Fig. 2).

In the molecular structure of lysozyme as determined by X-ray analysis the indole side-chain of Trp 62 is directly above that of Trp 63. It seems plausible that this arrangement leads to enhanced electron-donor properties analogous to those found for aromatic systems in cyclophanes [17].

Experimental Part

Materials. Chicken egg-white lysozyme (grade-1; lot nr. 110–8150) was obtained from *Sigma Chem. Comp.* and used without further purification. Lysozyme concentrations were determined spectrophotometrically based on a molar extinction of 37,913 $1 \cdot mol^{-1} \cdot cm^{-1}$. Samples of human calcitonine-(11–32)-dokosipeptide and complete human calcitonine were obtained by the courtesy of Dr. *W. Rittel* (*Ciba-Geigy* AG). A sample of ACTH-(1–24)-tetrakosipeptide was obtained from Dr. *R. Baumann*. All simple aromatic amino acid derivatives were obtained from *Fluka AG*, Buchs, Switzerland.

Paraquat. – 4,4'-Bipyridyl (10 mmol; *Fluka*) was reacted with methyl iodide (25 mmol) in dimethylformamide (25 ml) for 24 h at room temperature in the dark. The bright red N,N'-dimethyl-4,4'-dipyridylium diiodide was obtained in 98% yield by precipitation with ether. The diiodide was converted to the dichloride by stirring in water with an excess of freshly prepared silver chloride during 3 h in the dark. After filtration of the excess of silver chloride and of the silver iodide formed, the colourless solution of the dichloride was evaporated *in vacuo* at 30°. This gave the paraquat dichloride dihydrate in 95% yield. From the dihydrate the waterfree paraquat dichloride can be obtained as a slightly hygroscopic, white, crystalline material by drying over phosphorus pentoxide *in vacuo* at 50°.

NMR. spectra were measured at 28–29° on different *Varian* spectrometers: T-60; XL-100 (employing *Fourier*-transform to increase sensitivity), and HR-220.

Spectrophotometric measurements were carried out in teflon stoppered silica cells with 1 cm path at 20°. Variation of the absorbance with concentration was recorded with a *Zeiss* PMQ-II spectrophotometer; complete spectra were obtained with a *Beckman* Acta-V recording spectrophotometer.

Association constants were determined from the effect of increasing amounts of paraquat (0.01–0.5 M in 8 or 9 steps) on the CT. absorption of a solution containing about 0.001 M of the amino-acid derivative or the polypeptide. Mostly this was done by addition of solid paraquat to a cuvet containing the dilute solution of the peptide. The volume of the solution after addition of y mg of paraquat can be calculated from the starting volume in ml(V_0) by:

$$V_y = V_0 + y \cdot 0.0008$$

With lysozyme, the addition of solid paraquat leads to the formation of light-scattering particles, probably due to some denaturation of the protein at the crystal surface of paraquat. This problem was overcome by adding paraquat as a concentrated solution (1M).

The spectrophotometric data thus collected at various wavelengths were evaluated via the *Scatchard* equation [5] [18]:

$$\frac{A}{P_0 X_0} = -K \cdot \frac{A}{P_0} + K \cdot \varepsilon$$

A = absorbance measured (corrected for residual absorption of paraquat), X_0 = total concentration of paraquat (M), P_0 = total concentration of the amino acid or polypeptide, K = association constant ($1 \cdot mol^{-1}$), ε = molar extinction ($1 \cdot mol^{-1} \cdot cm^{-1}$).

From a linear least squares fit of A/P_0 versus (A/P_0X_0), K can be determined as the slope and $K \cdot \varepsilon$ as the intercept. Very good fit of the data to a linear *Scatchard* plot was obtained throughout, as indicated by the high correlation coefficients calculated. The results are compiled in Table 2.

Prof. Dr. *K. Wüthrich* is gratefully acknowledged for providing the NMR. facilities and for his stimulating discussion of the results. We thank Dr. *J. W. van Nispen* from the University of Nijmegen for conveying to us his results on the spectrophotometric binding studies of paraquat to ACTH-(1–24)-tetrakosipeptide prior to publication. For two of us (*J. W. V.* and *A. M. A. V.*) the present investigation was made possible by a grant of the *Netherlands Organisation for the Advancement of Pure Research* (*Z.W.O.*). Aid by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* is also gratefully acknowledged.

REFERENCES

[1] *G. Jori, M. Folin, G. Gennari, G. Galazzio & O. Buso,* Photochemistry and Photobiol. *19,* 419 (1974); *D. Creed,* Photochemistry and Photobiol. *19,* 459 (1974); *L. Stryer,* Science *162,* 526 (1968); *E. J. Gabbay,* J. Amer. chem. Soc. *91,* 5136 (1968); *O. H. Griffith & A. S. Wagonner,* Accounts chem. Res. *2,* 17 (1969).

[2] *R. Schwyzer & U. Ludescher,* Biochemistry *7,* 2514, 2519 (1968).

[3] *J. P. Carrión, B. Donzel, D. A. Deranleau, K. Esko, P. Moser & R. Schwyzer,* Peptides 1966 (North Holland Publ. Co., Amsterdam), 177 (1967); Helv. *51,* 459 (1968); *R. Schwyzer,* Proceedings of the Fourth International Congress on Pharmacology (Basel 1969), Vol. V, 196, Schwabe & Co., Basel (1970).

[4] a) *D. A. Deranleau & R. Schwyzer,* Biochemistry *9,* 126 (1970); b) *W. Boers & J. W. Verhoeven,* Biochim. biophys. Acta *328,* 1 (1973).

[5] *R. Foster,* 'Organic Charge Transfer Complexes', Academic Press, London 1969.

[6] *J. W. Verhoeven, I. P. Dirkx & Th. J. de Boer,* Tetrahedron *25,* 3395 (1969).

[7] *K. Wallenfels & W. Hanstein,* Liebigs Ann. Chem. *709,* 151 (1967).

[8] *W. R. Boon,* Chemistry & Ind. *782* (1965); *A. Nakahara & J. H. Wang,* J. phys. Chemistry *67,* 496 (1963).

[9] *A. Ledwith & D. H. Iles,* Chemistry in Britain *4,* 266 (1968); *A. Ledwith & H. J. Woods,* J. chem. Soc. (C) *1970,* 1442.

[10] *A. Masson,* Dissertation 5229, ETH-Zürich (1974).

[11] *R. Schwyzer & H. Kappeler,* Helv. *44,* 1991 (1961).

[12] *R. Schwyzer, P. Schiller, J.-L. Fauchere, G. Karlaganis & G. M. Pelican* in: 'Proceedings of the Second International Symposium on the Structure Activity Relationship of Protein and Polypeptide Hormones', p. 167, Liege 1971, ed. M. Margoulies & F. C. Greenwood, Excerpta Medica (1972).

[13] *J. W. van Nispen & G. I. Tesser,* Int. J. Peptide Protein Res., in press.

[14] *D. A. Deranleau, R. A. Bradshaw & R. Schwyzer,* Proc. Nat. Acad. Sci. USA *63,* 885 (1969); *R. A. Bradshaw & D. A. Deranleau,* Biochemistry *9,* 3310 (1970).

[15] *H. Sternlicht & D. Wilson,* Biochemistry *6,* 2881 (1967); *K. Wüthrich,* Chimia *24,* 409 (1970).

[16] *J. W. Verhoeven & R. Schwyzer,* Helv. *55,* 2572 (1972).

[17] *D. J. Cram & R. H. Bauer,* J. Amer. chem. Soc. *81,* 5971 (1959).

[18] *D. A. Deranleau,* J. Amer. chem. Soc. *91,* 4050 (1969).

273. Eine einfache Synthese von 2,2-Dimethyl-thietan

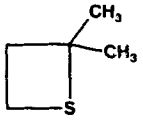
von **Carl Mayer**

Zentrale Forschung, Ciba-Geigy AG, 4000 Basel

(11. XI. 74)

Summary. A simple two step procedure for the synthesis of the naturally occurring 2,2-Dimethyl-thietan is given. Yield about 90%.

Für das von *Schildknecht & Wilz* [1] 1967 als Geruchstoff des Nerzes isolierte 2,2-Dimethyl-thietan (**1**) fehlte bisher eine einfache Darstellungsmethode. Die von



1