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INTERACTION OF TETRACYANOETHYLENE WITH INDOLES ON SILICA GEL AND CELLULOSE THIN-LAYER PLATES*

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SUMMARY

Tetracyanoethylene and indoles readily form dark coloured π-complexes in solution. In most instances the components of such complexes formed from indoles unsubstituted in the 3-position, interact on standing with the formation of 3-tricyanovinylindole derivatives. However the use of tetracyanoethylene as a spray reagent for detecting indoles on thin-layer plates leads exclusively to the formation of these 3-tricyanovinyl substituted products. The structures of some of these compounds have been confirmed using physical and chemical methods.

INTRODUCTION

Electron acceptor reagents have proved to be of considerable value in thin-layer chromatographic (TLC) procedures for the detection and subsequent identification by mass spectrometry of many electron-rich aromatic and heterocyclic compounds, including a number of biologically important indole derivatives, such as 3-indolylacetic acid and serotonin[1–5].

Previous work carried out in these laboratories and elsewhere has shown that a number of different electron acceptor reagents are suitable for this purpose[1–5]. Tetracyanoethylene (TCNE) is one example of a reagent of this type which has been widely used in this manner[1]. However whilst it is generally recognized that TCNE initially forms electron donor–acceptor complexes with indoles (*e.g.* Fig. 1, I) in solution[6], as shown by the rapid formation of deep blue-black coloured products in solution. The components of these complexes (*e.g.* Fig. 1, II) readily interact with each other to form 3-tricyanovinylindole derivatives (*e.g.* Fig. 1, III) by the elimination of hydrogen cyanide[6,7]. This reaction, which has been reported to be base catalyzed[8,9], occurs very readily in solution and in some instances even occurs in the solid state.

SAUSEN *et al.*[8] first reported the preparation of 3-tricyanovinylindole (Fig. 1, IIIc) in 1958, by the interaction of indole and TCNE in pyridine at room temperature using essentially the procedure described by HECKERT[10] for the preparation of some

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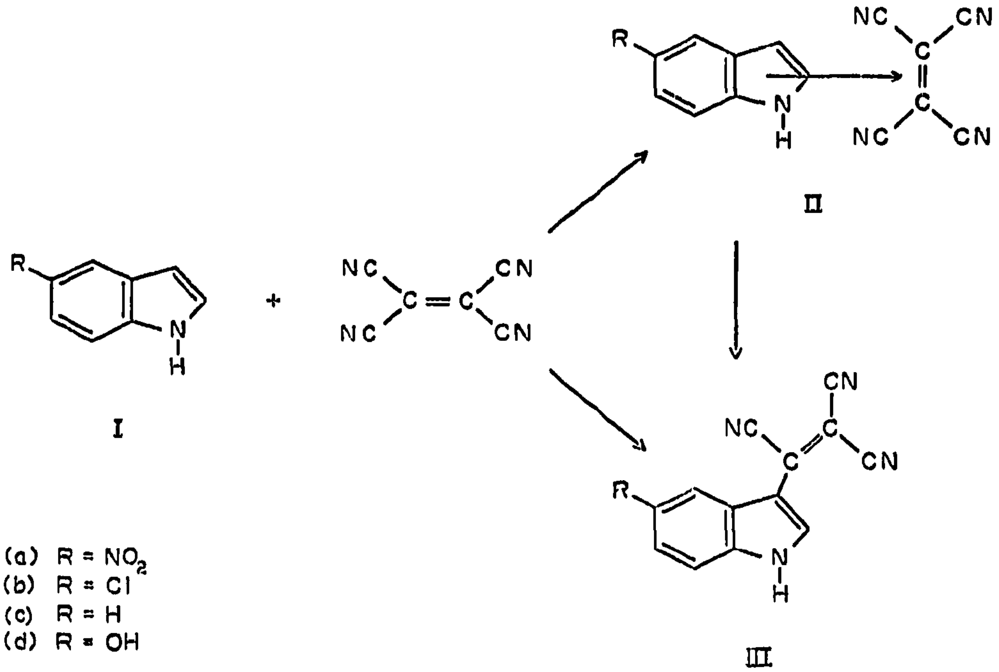


Fig. 1. Complex formation and reaction of TCNE with indoles.

related compounds. Shortly afterwards NOLAND *et al.* improved the overall yield of 3-tricyanovinylindole (Fig. 1, IIIc) to 92% by carrying out the reaction in boiling benzene in the presence of a small amount of pyridine[9].

In cases where the 3-position of the indole nucleus was blocked (*e.g.*, skatole *i.e.* 3-methylindole) tricyanovinylation of the indole nucleus was reported to occur on the nitrogen atom[9].

It had been noted in some preliminary experiments that colours obtained from some indole compounds on thin-layer plates, when sprayed with TCNE, were not the same as those observed in solution. This was particularly true of indole compounds with a free 3-position, but with 3-substituted indoles only dark blue-black colours were obtained both on the plate and in solution suggesting that in these cases complexation only had occurred.

This phenomenon was observed under "neutral" conditions, but in the presence of a small amount of pyridine the dark blue solution obtained by the interaction of a typical 3-substituted indole (3-indolylacetic acid) and TCNE was rapidly discharged, due to the formation of an orange-yellow compound in solution.

We now wish to report the formation of the 3-tricyanovinylindole derivatives from 5-substituted indoles, both in solution and on silica gel or cellulose layers.

MATERIALS AND METHODS

General

The melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The NMR spectra were obtained on a Varian A-60A instrument using tetramethylsilane as an internal reference. The mass spectra were obtained on a DuPont/C.E.C. 21-491 instrument. The reflectance spectra were obtained with the aid of a Farrand UV-visible chromatogram analyser.

Tetracyanoethylene and all the indole compounds used in this investigation were obtained from the Aldrich Chemical Co.

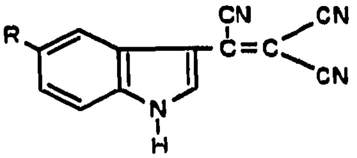
Chemical procedure (interaction of indoles with TCNE)

Preparation of 3-tricyanovinylindoles, from:

(a) Indole, 5-chloroindole and 5-nitroindole. The 3-tricyanovinylindole compounds (IIIa, IIIb, and IIIc) were prepared by mixing equimolar amounts of the relevant indole compounds (Ia, Ib, and Ic, respectively,) with TCNE in boiling saturated solutions in dichloromethane. The reaction mixtures contained in stoppered flasks were allowed to stand at room temperature for three weeks, after which time the residual solvent was removed *in vacuo* and the dark coloured residues recrystallized from ethyl acetate or ethyl acetate–*n*-hexane mixtures. The resulting crude solid 3-tricyanovinylindoles (IIIa, IIIb, and IIIc, respectively) were finally purified by preparative scale TLC. The physical data for these compounds are given in Tables I and II.

TABLE I

SOME 3-TRICYANOVINYLINDOLES: PHYSICAL PROPERTIES AND MICROANALYSES



3-Tricyano-vinylindole derivative	M.p. (°C)	Colour	R_F value[a]	Analysis					
				Found (%)			Calculated (%)		
				C	H	N	C	H	N
IIIa, R = NO_2	269	orange	0.12	58.83	1.85	26.40	59.31	1.91	26.63
IIIb, R = Cl	295	orange-brown	0.26	61.30	1.92	22.22	61.45	1.98	22.47
IIIc, R = H	275[b]	brick-red	—	—	—	—	—	—	—
IIId, R = OH	295	red-brown	0.30	65.85[c]	2.47	24.08	66.66	2.57	23.93

[a] TLC on Silica Gel G; development with benzene–ethyl acetate (10:3).
[b] Literature m.p.: 273–275° (ref. 6); 275–276° (ref. 8); 268–270° (ref. 9).
[c] It was not possible to obtain a completely satisfactory value for carbon in this instance.

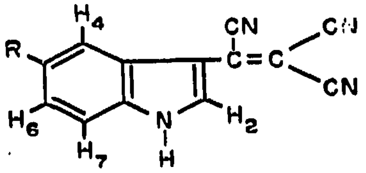
(b) 5-Hydroxyindole. All attempts to prepare 5-hydroxy-3-tricyanovinylindole (IIId) by the interaction of 5-hydroxyindole (Id) and TCNE in solution under the conditions described above were unsuccessful. However this product could be obtained by an adaptation of the method of SAUSEN *et al.*[8] or in the following manner.

A solution of 20 mg of 5-hydroxyindole (Id) in 10 ml of benzene was sprayed uniformly onto a Merck precoated Silica Gel G plate (20 × 20 cm; thickness, 0.25 mm). After the plate had been allowed to dry it was repeatedly sprayed with a solution of TCNE in dichloromethane. The silica carrying the product was removed from the plate and the product eluted from the silica with ethyl acetate. The crude 5-hydroxy-

TABLE II

NMR SPECTRAL DATA FOR SOME 3-TRICYANOVINYLINDOLES

The NMR spectra were measured in acetone-d_6 solution using tetramethylsilane as internal reference. (s) = singlet; (d) = doublet; (dd) = doublet of doublets.



3-Tricyanovinylindole derivative	H_2	H_4		H_6			H_7	
	δ value[a]	*δ value*[a]	*$J_{4,6}$ value*[b]	*δ value*[a]	*$J_{4,6}$ value*[b]	*$J_{6,7}$ value*[b]	*δ value*[a]	*$J_{6,7}$ value*[b]
IIIa, R = NO_2	8.95 (s)	9.17 (d)	2.0	8.30 (dd)	2.0	9.0	7.97 (d)	9.0
IIIb, R = Cl	8.73 (s)	8.07 (d)	2.0	7.43 (dd)	2.0	9.0	7.70 (d)	9.0
IIId, R = OH	8.73 (s)	7.70 (d)	2.0	7.00 (dd)	2.0	9.0	7.52 (d)	9.0

[a] δ = chemical shift in p.p.m.

[b] J = coupling constant in Hz.

3-tricyanovinylindole (IIId) obtained in this manner was purified by the method described above. Physical data for this compound are given in Tables I and II.

Preparation of complexes

The 3-indolylacetic acid–TCNE and indole–TCNE complexes were obtained as dark blue microcrystalline solids by the method previously described by HUTZINGER AND JAMIESON[4], with the following modification for the 3-indolylacetic acid complex.

A mixture of cold saturated solutions of 3-indolylacetic acid and TCNE in dichloromethane was stored at −20° and the dark blue microcrystalline complex (m.p. 147°) removed by filtration.

Analysis, calculated, for $C_{16}H_9N_5O_2$:C, 63.36; H, 2.99; N, 23.09%. Found: C, 62.78; H, 2.97; N, 23.41%.

Thin-layer chromatography

Plates. Commercially available Merck Silica Gel G or Merck cellulose plates (20 × 20 cm; thickness, 0.25 mm and 0.1 mm, respectively) were used for analytical separations. Preparative scale TLC was carried out on plates (100 × 20 cm; thickness, 0.9 mm) prepared in the laboratory using Merck Silica Gel G as the adsorbant.

Solvent. A benzene–ethyl acetate (10:3) solvent system was used in all cases.

Analytical procedure

The indole compound (10–20 µg) was applied to the plate, from solution in acetone. After development with the solvent system described above, the dried plates were sprayed with a 1% solution of TCNE in dichloromethane, and the colours developed by the spots due to the indole compounds noted directly after spraying. The colours observed are reported in Table I.

Mass spectrometry. Mass spectra were obtained using the standard probe for direct introduction of the sample into the ion source.

Reflectance spectra. Reflectance spectra were recorded by plotting reflectance with reference to background *versus* wavelength at 10-nm intervals between 420 and 600 nm using the single-beam mode of operation. The light source was a xenon lamp and a 1P28 photomultiplier tube was the detector.

The monochromator was removed from the analyzer leg for these measurements, as the monochromator on the exciter leg provided monochromatic light. An auxiliary filter (No. 3-73, sharp-cut at 400 nm) was used in the analyzer leg, with suitable slit aperture reducers appropriate to the phototube response at the various wavelengths (an 0.031-mm reducer was used in the range of optimum response, approx. 450–600 nm, while 0.062- and 0.125-min reducers were used outside this range). The 3/16–11/32 slit set was used for all measurements.

RESULTS AND DISCUSSION

The results of this study confirm the relative ease with which 3-tricyanovinylindoles (*cf.* III) are formed by the interaction of TCNE with indole compounds unsubstituted in the 3-position. However highly coloured electron donor–acceptor complexes are definitely formed initially in solution and these complexes can be isolated in the solid state, if the appropriate precautions are taken. This has now been confirmed in the case of indole itself. The dark blue complex which can be isolated in the solid state by allowing its solutions in dichloromethane to crystallize at —20° is however unstable even in the solid state. On standing at —20° the colour of the solid slowly changes from dark blue to orange indicating the formation of the 3-tricyanovinyl derivative. Nonetheless it was possible to obtain a mass spectrum of a freshly prepared sample of the complex, which clearly shows the presence of both species. Only ions derived from the individual components of the complex can be seen in the spectrum and there is no evidence of peaks at higher *m/e* values corresponding to 3-tricyanovinylindole (see Fig. 2).

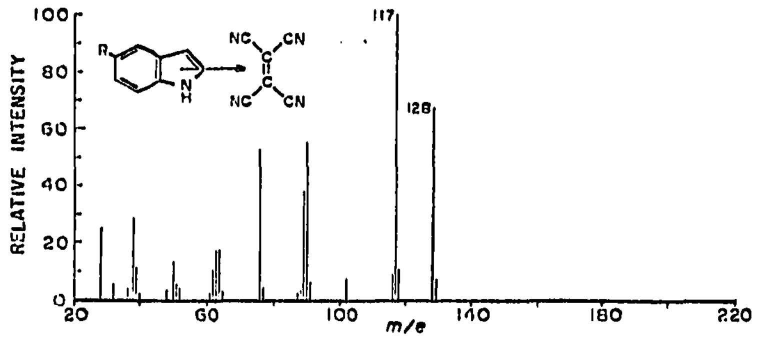


Fig. 2. 70-eV mass spectrum of the indole–TCNE complex at a probe temperature of 40°.

Fig. 3 shows the mass spectra of a few typical 3-tricyanovinylindoles (IIIa, IIIb, IIIc, and IIId). In all examples studied strong molecular ion peaks can be observed, and in no instances are peaks apparent due to the indole compound in question or TCNE.

Whilst it was possible to detect the "complex" stage in solution during the interaction of the indole compounds IIIa, IIIb, IIIc, and IIId, the 3-tricyanovinyl-

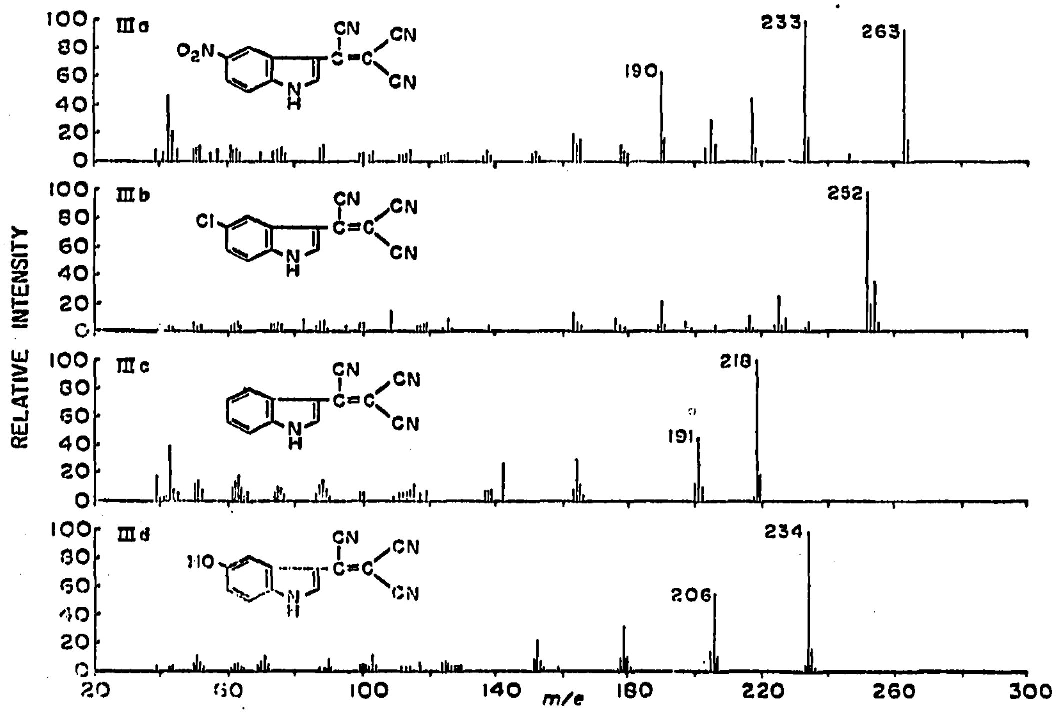


Fig. 3. 70-eV mass spectra of some 3-tricyanovinylindoles; probe temperatures: IIIa, 175°; IIIb, 135°; IIIc, 120°; IIId, 185°.

ation reaction occurred virtually instantaneously on silica or cellulose thin-layer plates. On spraying developed chromatoplates containing spots due to these indole compounds the very dark colours of the complexes were not seen, merely the orange to yellow colours of the 3-tricyanovinyl derivatives.

The NMR spectra of the 5-substituted 3-tricyanovinylindole derivatives confirm that the tricyanovinyl group is in the 3-position of the indole nucleus. The signals due to the H_2, H_4, H_6, and H_7 protons can be clearly seen and show the expected splitting patterns (see Table II). The characteristic signal for the H_3-indole proton, which is usually observed upfield from the H_2 proton and the protons in the 6-membered ring, is not seen in any of the spectra examined (*cf.* JARDINE AND BROWN[11]).

As mentioned earlier the decomposition of the indole–TCNE complexes to form tricyanovinyl derivatives does not occur as readily in the case of 3-substituted indoles, and the 3-indolylacetic acid–TCNE complex has been isolated as a relatively stable dark blue microcrystalline solid. Fig. 4 shows the mass spectrum obtained for this complex at two different temperatures, indicating that differential sublimation of the two components of the complex had occurred and that mass spectra due to both had been clearly obtained. No peaks at higher *m/e* values were observed indicating that no reaction of the two species had occurred. The formation of 1-tricyanovinyl derivatives from 3-substituted indoles has been reported[9] and it appears that this reaction is base catalyzed, since overspraying of blue chromatographic spots obtained from 3-indolylacetic acid with pyridine results in the discharge of the blue colour.

The reflectance spectra of the four 3-tricyanovinylindoles (IIIa, IIIb, IIIc, and IIId) have been measured on cellulose and the results are given in Fig. 5. The results clearly show that a broadening of the reflectance maximum towards longer wave-

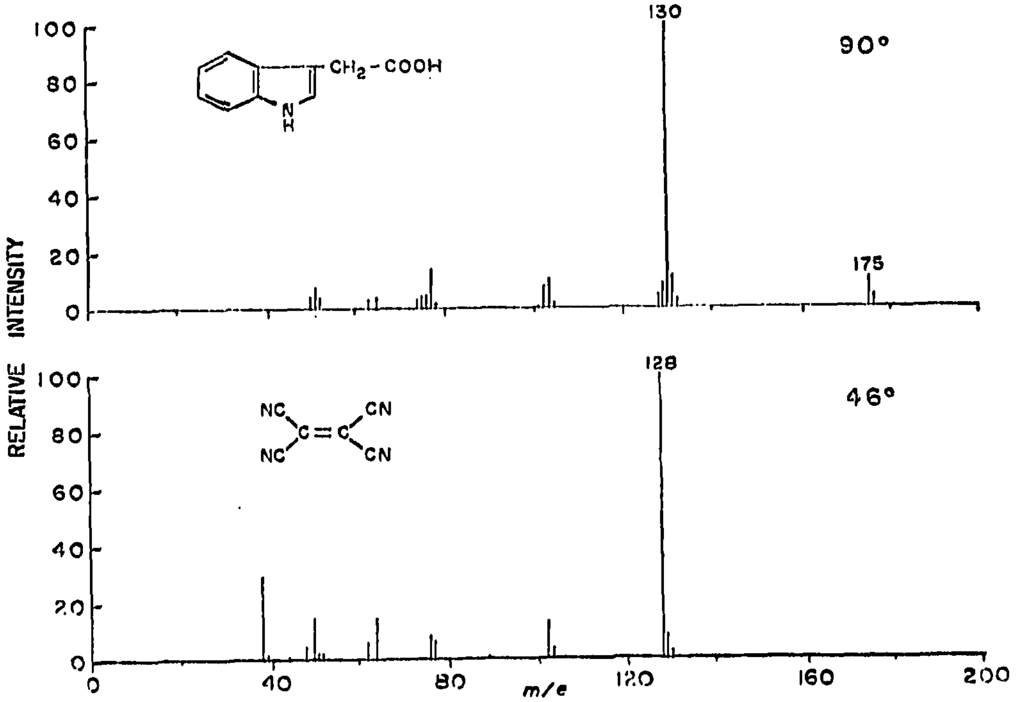


Fig. 4. 70-eV mass spectra of 3-indolylacetic acid–TCNE complex at probe temperatures shown.

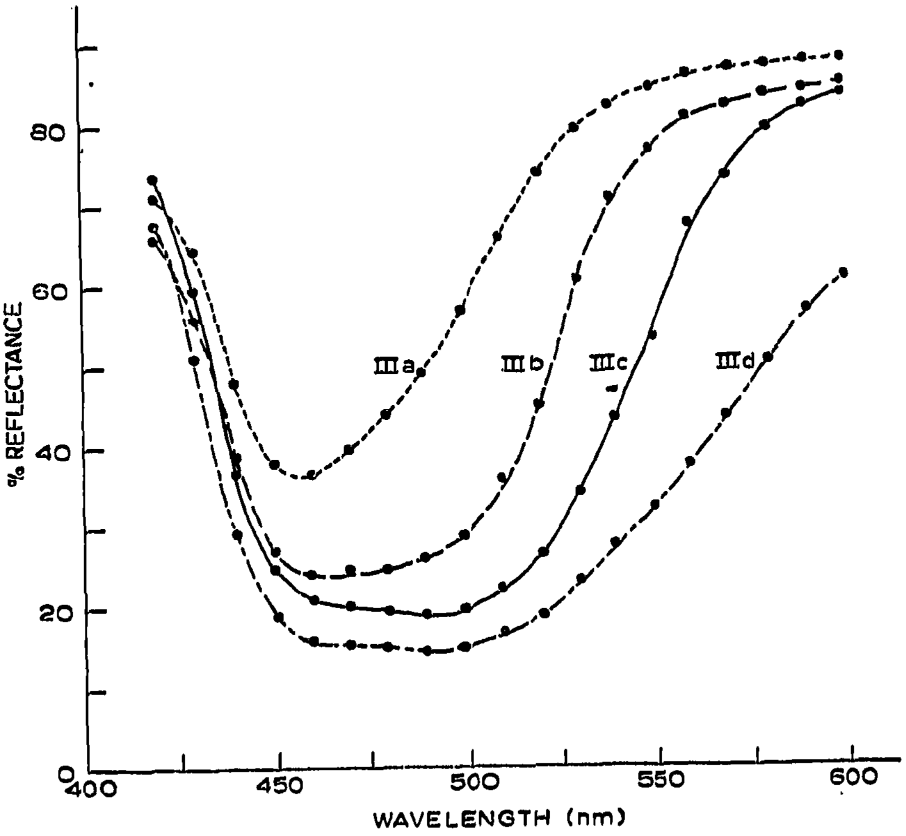


Fig. 5. Reflectance spectra of the 3-tricyanovinyl derivatives of 5-nitroindole, 5-chloroindole, indole, and 5-hydroxyindole (*i.e.* IIIa, IIIb, IIIc, IIId) formed *in situ* on cellulose.

lengths occurs with a decrease in the electron withdrawing properties of the substituent in the 5-position.

The reflectance spectra measurements could possibly form the basis of a quantitative method for the assay of indole compounds. A similar method, based on the formation of complexes between indole compounds and 2,4,7-trinitro-9-fluorenone has already been proposed[5].

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