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INTERACTIONS BETWEEN TETRACYANOETHYLENE AND *RAUWOLFIA* ALKALOIDS

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Interactions of the *Rauwolfia* alkaloids yohimbine, corynanthine, ajmalicine and reserpine with tetracyanoethylene were investigated by UV–visible spectroscopy. The results suggest the instantaneous formation of blue complexes whose thermodynamic and spectroscopic properties closely resemble those of 1:1 charge-transfer complexes of tetracyanoethylene with indole derivatives. The complexes are stable when the piperidinic nitrogen atom of the alkaloids is protonated or methylated. In contrast, those of the free bases rapidly decompose to give the corresponding 3,4-dehydro derivatives. The kinetics of these reactions have been studied and a mechanism is proposed.

INTRODUCTION

Rauwolfia alkaloids (RA) constitute a group of indole alkaloids which have long attracted interest on account of their biological activities, some of them being of therapeutic importance as sedative and antihypertensive agents.[1,2] Although the precise mode of action of these compounds has not been elucidated, they seem to be related to the presence of a tetrahydro-β-carboline nucleus as an integral part of the *Rauwolfia* skeleton. In fact, β-carbolines are known to interact with a variety of neuromodulators and neurotransmitters of the central nervous system.[3–5] More recently, the discovery that RA can act as cytotoxic or anticancer agents has stimulated new interest in these alkaloids.[6,7]

The formation of molecular complexes with biological acceptors has been suggested for the mechanism of action of these drugs.[8–10] In particular, intermolecular charge-transfer complexes (CTC) among β-carbolines and various biological acceptors have been claimed.[11,12] However, the ability of RA to form CTC has scarcely been investigated,[13,14] in spite of the well documented capacity of the parent indoles to act as electron donors in this kind of complex.[15]

In connection with our interest in the reactivity of β-carboline derivatives, we have recently initiated a research programme focused on this question. The well known acceptor tetracyanoethylene (TCE)[16] was chosen to study the donor properties of RA possessing a tetrahydro-β-carboline nucleus in their structures and this paper reports the kinetic and thermodynamic results derived from that study. In order to account for the different structural characteristics of the *Rauwolfia* skeleton, the alkaloids shown in Scheme 1 were selected.

EXPERIMENTAL

Sample of the alkaloids of the highest purity available were a gift from Boehringer or purchased from Aldrich Chemie and were used without further purification. Npiperidinic-methyl derivatives were prepared as sulphate salts using literature methods.[17] TCE (Aldrich) was recrystallized from dichloromethane. Stock solutions of the reagents were prepared in dichloromethane. TCE solutions were prepared daily and the alkaloid solutions were protected from light and frequently renewed.

Spectral analyses of the products were performed with a Perkin-Elmer Lambda 5 spectrophotometer. Rapid scans of the reaction mixtures were run on a Hewlett-Packard 8452A spectrophotometer with a diode-array detection system. Kinetic measurements were carried out on an LKB-Ultrospec Plus spectrophotometer equipped with a rapid mixing system. An interfaced Multitech Acer 500 computer was used to control the spectrophotometric system, the uptake of the absorbance–time data and its processing. In all the static and kinetic measurements the temperature control was better than ±0·1 °C.

RESULTS AND DISCUSSION

The spectrophotometric analysis of the evolution with time of the reaction mixtures can be summarized as follows. On mixing the reagents, royal blue products are

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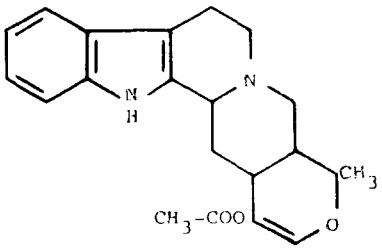
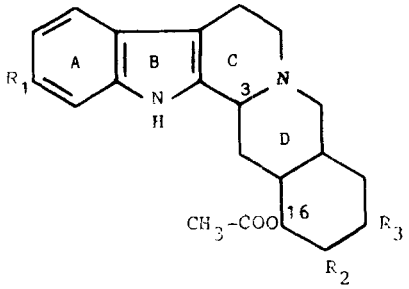


AJMALICINE: C-3 (H-axial), C/D (trans)



YOHIMBINE: C-3 (H-equatorial), C/D (trans), C-16 (H-axial), R_1=R_3=H, R_2=OH

RESERPINE : C-3 (H-equatorial), C/D (cis), C-16 (H-equatorial), R_1=R_2=OCH_3, R_3=TMB

TMB= trimethoxybenzoic acid

CORYNANTHINE: C-3 (H-equatorial), C/D (trans), C-16 (H-equatorial), R_1=R_3=H, R_2=OH

Scheme 1

instantaneously formed. The spectral and thermodynamic properties of these compounds as will be shown later, strongly support the formation of 1 : 1 CTC in this step. The stabilities of the complexes are markedly influenced by the state of the piperidinic nitrogen atom in the tetrahydro-β-carboline nucleus of the alkaloid. Thus, the complexes of Npi-protonated or Npi-methylated alkaloids are stable for several hours, whereas those of the free bases are very unstable.

In the latter cases the blue colour rapidly disappears and yellow–green products are formed in a few seconds. Figure 1 for ajmalicine is an example of their behaviour. These products were identified as the corresponding 3,4-dehydro derivatives of the alkaloids by spectral comparison (UV-visible, IR and fluorescence) with solutions of authentic specimens prepared by reaction of the alkaloids with nitrous acid[18] and subsequent extraction with dichloromethane.

On longer time scale, further reaction products are slowly formed. Chromatographic analyses of the reaction mixtures show, in addition to the didehydro derivatives, other products with absorption maxima at 412 and 390 nm. The identification of these products was not attempted.

In order to obtain information regarding the nature of the blue products, we attempted a thermodynamic study of their formation reactions. Owing to the instability of most of them and solubility problems, this study could only be carried out with a representative

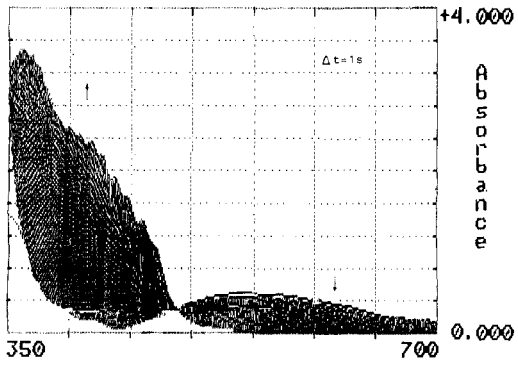


Figure 1. Spectral analysis of the evolution with time of a mixture of ajmalicine (3×10^{-3} M) and TCE (0·05 M)

Table 1. Spectral and thermodynamic characteristic of CTC

Substrate	λ_{max}CTC (nm)	[Substrate] (M)	[TCE] (M)	t(°C)	ε_{AD} (l mol^{-1} cm^{-1})	K (l mol^{-1})	ΔH_f(kcal mol^{-1})	ΔS_f(cal mol^{-1} K^{-1})
2,3-DMI	700	5×10^{-4}	0·01–0·1	15·5	2160 ± 114	18·1 ± 1·8	–	–
				20·0	2365 ± 116	14·2 ± 1·2	–	–
				25·0	2390 ± 85	12·2 ± 0·7	−7·28	−19·5
				30·0	2500 ± 117	9·7 ± 1·0	–	–
THC	560	0·01–0·2	4×10^{-4}	15·5	1890 ± 96	22·6 ± 2·0	–	–
				19·5	1716 ± 63	19·4 ± 1·9	–	–
				25·0	1695 ± 88	16·5 ± 1·6	−5·79	−13·8
				30·0	1820 ± 133	13·7 ± 2·1	–	–
PY	525	$1·5 \times 10^{-3}$	0·04–0·1	10·0	2688[a]		–	–
				15·0	2394[a]		–	–
				20·0	2157[a]		–	–
				24·0	1990[a]		−3·78	–

[a]These values correspond to $K_{AD}\varepsilon_{AD}$.

substrate, Npi-protonated yohimbine (PY). For purposes of comparison, the model compounds, 2,3-dimethylindole (2,3-DMI) and tetrahydrocarbazole (THC) were also included in this study.

The apparent formation constants, defined as

$$K_{AD} = \frac{[CTC]}{[RA]\,[TCE]} \quad (1)$$

where determined spectrophotometrically by fitting the absorbance–concentration data obtained at the maximum absorption wavelength of the complexes to equation (2) using a non-linear optimization method based on the algorithm of Hooke and Jeeves:[19]

$$A_t = \varepsilon_A(C_A^0 - C_{AD}) + \varepsilon_D(C_D^0 - C_{AD}) + \varepsilon_{AD}C_{AD} \quad (2)$$

where C_A^0 and C_D^0 are the initial concentrations of the acceptor and donor, respectively, and C_{AD} is the product concentration.

Unfortunately, it was impossible to obtain individual K_{AD} and ε_{AD} values for the Npi-protonated yohimbine complex, as under our experimental conditions (10–24 °C) the solubilities of the reactants were insufficient to obtain the concentrations of the complex necessary to achieve the numerical calculations. Therefore, only the products $K_{AD}\varepsilon_{AD}$ could be evaluated with adequate precision. The values of K_{AD} and ε_{AD} at different temperatures are given in Table 1.

The enthalpies and entropies reported in Table 1 were obtained from van't Hoff equation plots and from the standard thermodynamic equations $\Delta G = \Delta H - T\Delta S$ and $\Delta G = -RT \ln K$. A perusal of the data in Table 1 reveals the similarities between all these complexes and supports the formation of 1 : 1 CTC between RA and TCE with similar characteristics to those of typical indoles.

KINETIC STUDIES

To obtain further insight into the interactions between RA and TCE, we also carried out a kinetic study on the dehydroderivative formation reactions. For this purpose, the representative alkaloids corynanthine, ajmalicine and reserpine were selected. The reactions were followed by continuously monitoring the absorbances of the reaction mixtures at the absorption maxima of the products. Pseudo-first-order conditions with TCE in excess were always used and rate constants calculated from a non-linear fitting of the absorbance–time data to the equation

$$A_t = A_\infty + (A_0 - A_\infty)\exp(-k_{obs}t) \quad (3)$$

All experiments were performed in duplicate and the corresponding experimental rate constants always agreed to within 5%. The values of k_{obs} at various initial concentrations of TCE and 25 °C are given in Table 2.

Table 2. Pseudo-first-order rate constants at 25 °C

	$k_{obs} \times 10^2$(s^{-1})		
[TCE] (10^{-4} M)	Ajmalicine[a]	Corynanthine[b]	Reserpine[c]
1		0·66	
2	0·19		0·82
2·5		1·12	
4	0·36	1·69	1·17
5		2·11	
6	0·58		1·57
7·5		3·06	
8	0·75		1·96
10	0·91	3·48	2·44
20	1·60	5·62	3·51
30	2·54	7·12	4·28
40	3·48	8·11	4·66
50		9·00	5·16

[a][Ajmalicine]$_0 = 3·5 \times 10^{-5}$ M; $\lambda_{exp} = 360$ nm.
[b][Corynanthine]$_0 = 5 \times 10^{-5}$ M; $\lambda_{exp} = 360$ nm.
[c][Reserpine]$_0 = 5 \times 10^{-5}$ M; $\lambda_{exp} = 402$ nm.

The dependence of k_{obs} on TCE concentration (Fig. 2) for corynanthine–TCE and reserpine–TCE

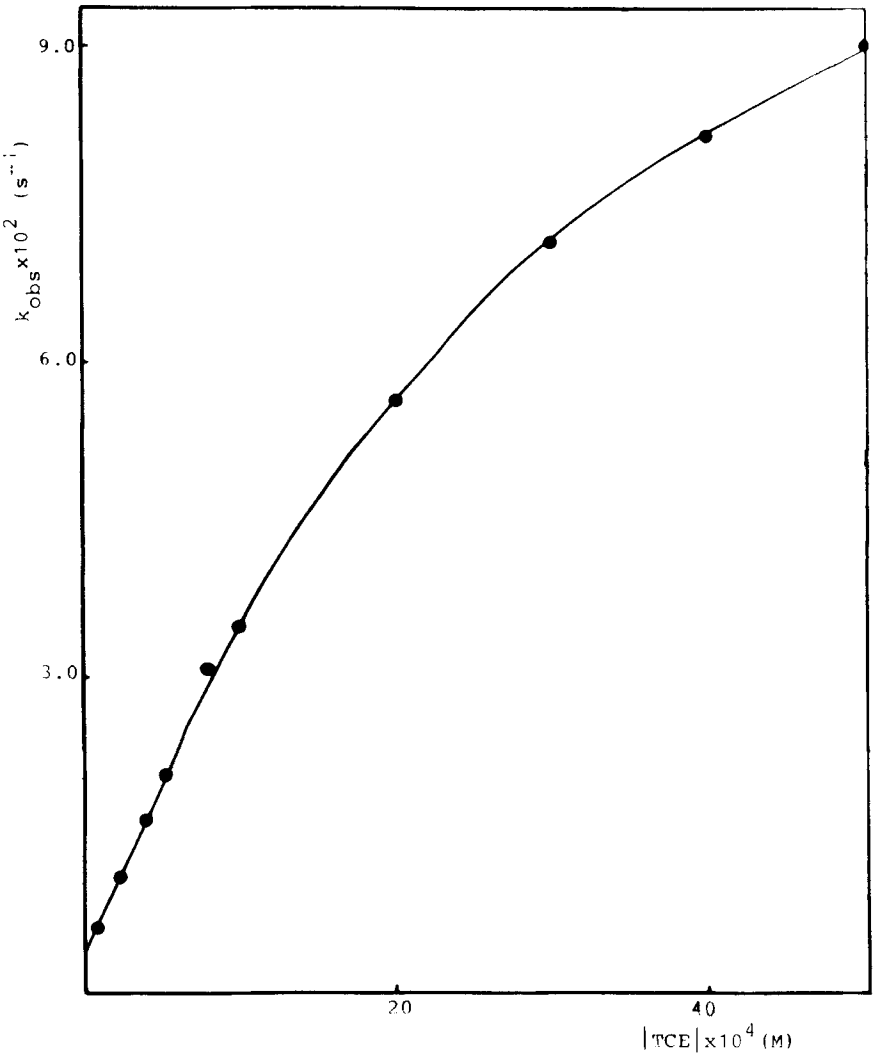


Figure 2. Dependence of k_{obs} at 25 °C on TCE concentration for the corynanthine–TCE reaction

reactions shows initially an almost linear increase but tends to level off at high concentrations of TCE. The existence of a small but significant intercept on the ordinate is also observed.

These results can be expressed mathematically by the general equation

$$k_{obs} = a + \frac{b[TCE]}{1 + c[TCE]} \quad (4)$$

The data for the ajmalicine–TCE reaction can also be fitted to this equation with $a = 0$ and $1 \gg c[TCE]$. The values of the kinetic parameters in equation (4) are summarized in Table 3.

It must be noted that the second term of the right-hand side of equation (4) indicates the accumulation of a TCE–alkaloid complex. However, it is noteworthy that the values obtained for c are greater than those reasonably expected for K_{AD}. Therefore, another intermediate apart from CTC is required in order to explain the experimental results.

Table 3. Kinetic parameters of equation (4) at 25 °C

Substrate	a (s^{-1})	b ($l\,mol^{-1}\,s^{-1}$)	c ($l\,mol^{-1}$)
Ajmalicine	–	8·46	–
Corynanthine	$2{\cdot}32 \times 10^{-3}$	43·48	358·7
Reserpine	$3{\cdot}83 \times 10^{-3}$	22·70	240·6

REACTION MECHANISM

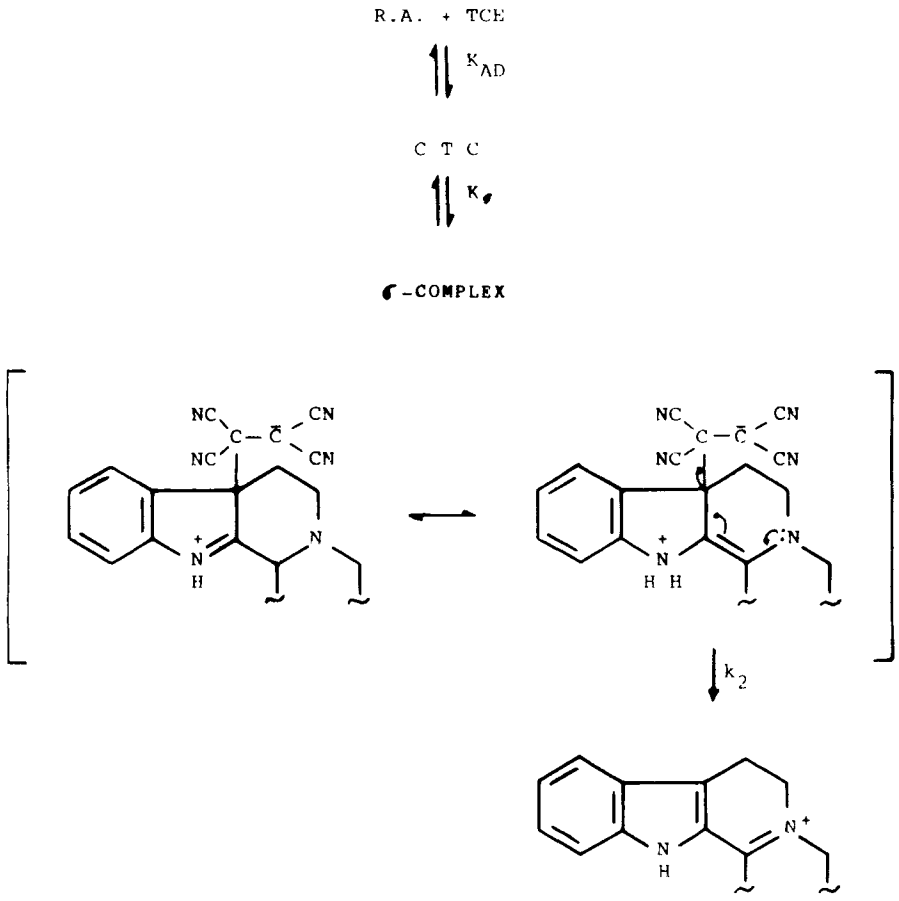
All the above thermodynamic and kinetic observations led us to suggest the provisional reaction mechanism in Scheme 2. It is based on the reorganization pathways proposed for the CTCs of indole and anilines[20,21] and on the typical reaction patterns of tetrahydro-β-carbolines.[17,22-24]

This mechanism involves a prior equilibrium step to form the CTC, which rearranges to give a sort of σ-complex. Taking into account the nature of the substrates, it seems reasonable to expect the indoleninic structure shown in the mechanistic Scheme 2 for this intermediate. It should be noted that the related intermediate in the reorganization process of the *N*,*N*-dimethylaniline–TCE complex has been isolated and characterized.[25] The decomposition of the indoleninic intermediate to give 3,4-dehydro compounds is typical of electrophilic substitution of tetrahydro-β-carbolines and it is the most distinctive pattern of reactivity of these compounds with respect to simpler indoles. It is evident that this rearrangement must necessarily be assisted by an electron pair from the piperidinic nitrogen atom. This explains the stability of the CTC when this atom is protonated or methylated.

According to the proposed mechanism, the following expression can be derived for k_{obs}:

$$k_{obs} = \frac{k_2 K_{AD} K_\sigma [TCE]}{1 + K_{AD} K_\sigma [TCE]} \quad (5)$$

It is clear that this mechanism only accounts for some aspects of the reactions. In fact, it does not explain the existence of the term independent of TCE concentration observed in the experimental rate law. Although several mechanistic alternatives can be proposed for interpreting this independent term, we suggest as the simplest explanation the existence of a parallel reaction pathway without involving CTC formation. TCE is an unusually strong electrophilic reagent capable of vinylic



Scheme 2

substitutions. However, it is not evident why this path might be independent of TCE concentration. This reaction probably takes place with the formation of an intermediate of similar structure to that of the proposed σ-complex, the rate law in this case also being similar but with $1 \ll c'$ [TCE].

On the other hand, the different reactivities of the alkaloids can be ascribed to stereochemical factors.[26,27] Thus, those alkaloids which possess an equatorial hydrogen atom on C-3, such as corynanthine and reserpine (see Scheme 1), show a close kinetic behaviour in spite of their different C/D ring junction conformations (*trans* and *cis*, respectively). In contrast, ajmalicine, with the same C/D conformation as corynanthine but different C-3 hydrogen configuration (axial and equatorial, respectively), shows a very different kinetic behaviour. It is evident, therefore, that the stereochemistry affects both the rate and equilibrium constants in equations (4) and (5). However, the experimental conditions do not allow the individual values of many of these parameters to be obtained and, therefore, preclude a more detailed discussion on the mechanistic implications of these factors. We expect that further work on this topic would help to clarify this point.

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