Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of the antimalarial and cytotoxic alkaloid cryptolepine complexed with the DNA fragment d(CCTAGG)$_2$

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Received 4 July 2001
Accepted 7 November 2001

Crystals of the indoloquinoline alkaloid cryptolepine complexed with the DNA fragment d(CCTAGG)$_2$ have been grown by the hanging-drop technique at 293 K using ammonium sulfate as the precipitating agent. Over a period of three weeks, yellow tapering bullet-shaped crystals grew to maximum dimensions of 0.2 × 0.1 × 0.1 mm. The crystals belong to space group $P6_4$, with unit-cell parameters $a = b = 29.960$, $c = 39.64$ Å, $\alpha = \beta = 90$, $\gamma = 120°$, and diffract to 1.4 Å.

1. Introduction

Cryptolepine (Fig. 1) [5-methylquinolo-(2′,3′,3,2)-indole] is an indoloquinoline alkaloid isolated from the roots of the West African shrub *Cryptolepis sanguinolenta*. This natural product was first isolated from the roots of *C. triangularis* collected in Kisantu (Democratic Republic of Congo). Extracts of the roots of *C. sanguinolenta* are used clinically in Ghana for the treatment of malaria (Boye & Ampofo, 1983). Extracts have also been used as a remedy against colic, as a stomach ulcer tonic and cryptolepine itself has been found to produce a variety of pharmacological effects; these include hypotensive and antipyretic properties (Raymond-Hamet, 1937, 1938; Noamesi & Bamgbose, 1980), presynaptic α-adrenoreceptor blocking action (Noamesi & Bamgbose, 1982), anti-muscarinic properties (Rauwald *et al.*, 1992), anti-inflammatory properties (Bamgbose & Noamesi, 1981) and antibacterial effects (Boakye-Yiadom & Heman-Ackah, 1979; Paulo, Duarte *et al.*, 1994; Paulo, Pimentel *et al.*, 1994; Cimanga *et al.*, 1996).

Cryptolepine has potent *in vitro* activity against malaria parasites (*Plasmodium falciparum*) (Kirby *et al.*, 1995; Grellier *et al.*, 1996; Wright *et al.*, 1996; Cimanga *et al.*, 1997) and possesses cytotoxic activity, inhibiting DNA synthesis in B16 melanoma cells (Bonjean *et al.*, 1998). The compound has also been shown to form a complex with haematin in cell-free systems, suggesting that it has a quinine-like mode of action (Wright *et al.*, 1996). An understanding of the way in which cryptolepine combines with DNA will assist in the rational design of antimalarial compounds which inhibit β-haematin formation but which, unlike cryptolepine, do not intercalate into DNA. Derivatives of cryptolepine are currently being evaluated as leads to selective antimalarial agents (Wright *et al.*, 1997). More recently, the results of various studies (Bonjean *et al.*, 1998) have revealed that the alkaloid binds tightly to DNA and behaves as a typical intercalating agent. It was found that the drug interacts preferentially with GC-rich sequences and discriminates against homo-oligomeric runs of A and T. The study also led to the discovery that cryptolepine is a potent topoisomerase II inhibitor and a promising antitumour agent. It stabilizes topoisomerase II–DNA covalent complexes and stimulates the cutting of DNA at a subset of pre-existing topoisomerase II cleavage sites. The intercalating properties of cryptolepine are worthy of investigation as this compound may be a potential lead to new anticancer drugs. The determination of its structure was based on chemical reactions of a degradative nature and on ultraviolet spectroscopy in neutral and basic media. ^{1}H NMR, ^{13}C NMR and MS data have confirmed the proposed structure (Dwuma-Badu *et al.*, 1978; Ablordeppey *et al.*, 1990; Tackie *et al.*, 1991). A recent crystallographic study of the cryptolepine–tetraphenyl borate complex has also confirmed the structure and revealed a unique packing propensity (Wright *et al.*, 1999).

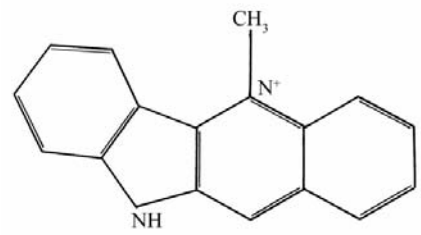

Figure 1
Structure of cryptolepine [5-methylquinolo-(2′,3′,3,2)-indole].

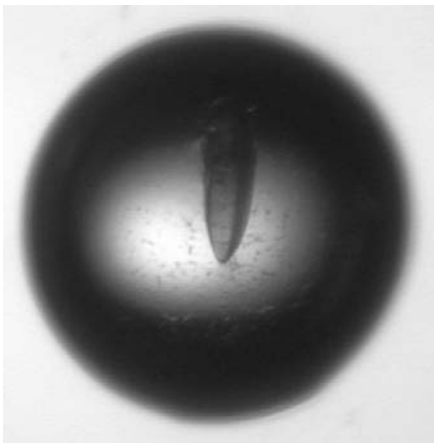

Figure 2
Crystal of the cryptolepine–$(CCTAGG)_2$ complex used for data collection.

2. Methods and results

Cryptolepine was isolated from the roots of *C. sanguinolenta* as described in detail elswhere (Wright *et al.*, 1996). Crystallization conditions were screened using the Natrix Screen (Hampton Research, USA) and the Nucleic Acid Mini Screen (Berger *et al.*, 1996). Crystals were grown at 293 K by the hanging-drop vapour-diffusion technique using Linbro multiwell tissue-culture plates. Crystals grew to a maximum size after about three weeks on mixing 0.5 µl 5 m*M* cryptolepine hydrochloride and 0.5 µl 3 m*M* $d(CCTAGG)_2$ with either 1.0 µl of crystallization solution containing 5 m*M* magnesium acetate, 25 m*M* MES pH 6.5 and 1.25 *M* ammonium sulfate or 2 µl of crystallization solution containing 5 m*M* magnesium sulfate, 25 m*M* sodium cacodylate pH 6.5 and 1 *M* ammonium sulfate. Yellow bullet-shaped crystals grew to maximum dimensions of 0.2 × 0.1 × 0.1 mm (Fig. 2) and diffracted weakly on an in-house rotating-anode generator. Synchrotron radiation was therefore essential for the success of this project. Crystals were flash-frozen in a stream of evaporating liquid nitrogen at 120 K. Diffraction data were collected at EMBL beamline BW7A (Hamburg, Germany) to 1.4 Å resolution using a MAR Research image-plate detector and a wavelength of 1.1 Å (see Table 1).

Table 1
Summary of the cryptolepine–DNA data set obtained at cryogenic temperature.

Values in parentheses are for the last resolution shell (1.45–1.40 Å).

Unit-cell parameters (Å, °)	$a = b = 29.96$, $c = 39.647$, $\alpha = \beta = 90$, $\gamma = 120$
Space group	$P6_4$
Resolution of data (Å)	1.4
No. of data collected	26108
No. of unique data	3356
Completeness (%)	87.3 (73.2)
$R_{\rm merge}$† (%)	3.8 (33)
Mean $\langle I/\sigma(I)\rangle$	29.1 (2.5)

† $R_{\rm merge}\ (I) = \sum_h \sum_i |I_i - I| / \sum_h \sum_i I$, where I is the mean intensity of i reflections h.

Determination of unit-cell parameters and space group and the integration of reflection intensities were performed using *DENZO* (Otwinowski, 1993) and the data were scaled using *SCALEPACK* (Otwinowski, 1993). The structure determination of the cryptolepine–$d(CCTAGG)_2$ complex is in progress.

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