

¹³C NMR AND OTHER SPECTRAL DATA OF 4-METHYLTHIOCANTHIN-6-ONE FROM *QUASSIA AFRICANA*

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ABSTRACT. The rare alkaloid 4-methylthiocanthin-6-one has been isolated from the root bark of *Quassia africana* (Simaroubaceae) together with 5-methoxycanthin-6-one. The MS, ¹H NMR and ¹³C NMR data for 4-methylthiocanthin-6-one are reported herein for the first time. In particular, unambiguous assignment of the ¹³C NMR chemical shifts have been made using 1D and 2D NMR experiments.

INTRODUCTION

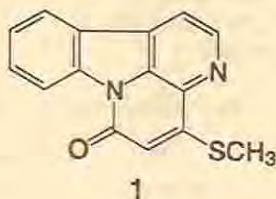
Quassia africana Baillon (Simaroubaceae) is an undergrowth wild shrub of the equatorial rain forest occurring from Cameroon to Angola. A decoction of the root bark is used as a febrifuge and analgesic in abdominal pains and tooth-ache. The root bark is also a household remedy against intestinal worms in Cameroon and Congo [1]. Quassinoids [2] and indole alkaloids [3] have previously been reported from *Q. africana*. In the course of our continuing search for plant filaricides [4,5], the methanol extract of *Q. africana* was found to exhibit significant *in vitro* filaricidal activity against *Onchocerca volvulus* microfilariae. Bio-assay directed fractionation of the active extract has led to the isolation and characterization of two compounds which were partially responsible for the *in vitro* activity of the methanol extract. These compounds were found to be the known indole alkaloids, 5-methoxycanthin-6-one [6] and 4-methylthiocanthin-6-one (1) [7]. This paper describes the isolation and spectroscopic characterization of the latter compound (1) which was isolated from *Pentaceras australis* (Rutaceae) and synthesized 40 years ago [7] but for which no spectral data (MS, ¹H NMR and ¹³C NMR) exist.

RESULTS AND DISCUSSION

The crude MeOH extract was dispersed in H₂O and the aqueous suspension was extracted with Et₂O and EtOAc. Each of these fractions was monitored by the previously described [4] assay for filaricidal activity. The Et₂O extract showed marginal activity with most of the MeOH-extract activity remaining in the H₂O-soluble fraction. Separation of the Et₂O extract by silica gel column chromatography and preparative TLC afforded 5-methoxycanthin-6-one and 4-methylthiocanthin-6-one (1).

Compound 1, pale yellow needles, had the molecular formula C₁₀H₁₅N₂OS from ¹³C NMR and elemental analyses. This formula was also corroborated by the EIMS which

showed an $[M]^+$ at m/z 266 (100%). The IR spectrum displayed bands at 1665 and 1638 cm^{-1} suggestive of an α,β -unsaturated lactam in addition to strong aromatic bands between 1620 and 1515 cm^{-1} . The UV spectrum was characteristic of a canthin-6-one [8,9] and was identical with that reported for 4-methylthiocanthin-6-one [7]. A detailed analysis of the MS and the 1D and 2D NMR spectral data (*vide infra*) clearly indicated that compound **1** was 4-methylthiocanthin-6-one.



The ^1H NMR spectrum of **1** was well-resolved and is summarized in Table 1. The data were interpreted on the basis of published ^1H NMR data for known canthin-6-ones [8,9], and by analysis of its ^1H - ^1H COSY spectrum. Of particular significance was the up-field three-proton signal at δ 2.60 which was attributed to the 4-thiomethyl group following the assignment of the one-proton singlet at δ 6.55 ppm to H-5 by analogy to the chemical shift of this proton in 4-methoxycanthin-6-one [10].

Table 1. NMR data^a of 4-methylthiocanthin-6-one (**1**).

Position	$^{13}\text{C}^b$	$^1\text{H}^c$ (J in Hz)	Direct and Long range correlation
1	117.00, <i>d</i>	8.00, <i>d</i> (5.0)	H-1, H-2
2	144.83, <i>d</i>	8.00, <i>d</i> (5.0)	H-2
4	155.05, <i>s</i>	-	SMe-4
5	117.34, <i>d</i>	6.55, <i>s</i>	H-5
6	158.20, <i>s</i>	-	
8	117.00, <i>d</i>	8.65, <i>d</i> (8)	H-8
9	130.89, <i>d</i>	7.70, <i>ddd</i> (8,8,2)	H-11
10	125.24, <i>d</i>	7.50, <i>ddd</i> (8,8,2)	H-10 ^d (only observed in ^1H - ^{13}C COSY)
11	122.64, <i>d</i>	8.15, <i>d</i> (8)	H-11,
12	124.12, <i>s</i>	-	
13	139.20, <i>s</i>	-	H-11
14	130.89, <i>s</i>	-	H-1, H-2
15	121.12, <i>s</i>	-	
16	135.02, <i>s</i>	-	
4-SMe	13.85, <i>q</i>	2.60, <i>s</i>	H-5, H-2

^a in CDCl_3 ; Bruker AMX-300 spectrometer; Chemical shift referred to CHCl_3 at δ 7.26 and to CDCl_3 at δ 77.00.

^bAssigned on the basis of the ^1H - ^{13}C NMR COSY and the COLOC spectra and published ^{13}C -NMR spectral data of well known canthinones.

^cAssigned on the basis of published ^1H NMR spectral data for known canthin-6-ones and by analysis of the ^1H - ^1H COSY spectrum.

^dObtained from the COLOC spectrum.

In the study of the ^{13}C NMR spectrum of **1** (Table 1) it was found that very few reports exist on the ^{13}C NMR data of the over 30 canthin-6-one alkaloids known to date [11]. Apart from the work of Ohmato and Koike [11] who unambiguously assigned the ^{13}C chemical shifts of four members of this group including the parent canthin-6-one by using spin-spin coupling between ^{13}C and ^1H , and that of Kinghorn *et al.* [9] who employed a variety of 1D and 2D techniques including selective INEPT and ^1H - ^{13}C COLOC experiments in their own work, no other reliable ^{13}C assignments exist in the literature. In this study, the ^{13}C chemical shifts of all the proton-bearing carbons were assigned using ^1H - ^{13}C COSY experiments while long range heteronuclear COLOC experiments enabled the assignments of those of the quaternary carbons. These unambiguous assignments with the pertinent long-range correlations are summarized in Table 1.

The EIMS fragmentation pattern of 4-methylthiocanthin-6-one (**1**) was also similar to that of other methoxycanthin-6-ones [9,10,12]. It was however noted that the $[\text{M}+1]^+$ peak of **1** at m/z 267 was relatively more intense (26%) than in 4- and 5-methoxycanthin-6-ones. This could be a diagnostic feature of methylthiocanthinones.

This is the first report of **1** from *Quassia africana* and the second report of this alkaloid from a plant source. 4-Methylthiocanthin-6-one was weakly active against *Onchocerca volvulus* microfilariae. The result of the activity will be published elsewhere.

EXPERIMENTAL

General: CC: Si gel; TLC: Si gel using cyclohexane-EtOAc (3:2) or CHCl_3 -MeOH (98:2).

Plant material The roots of *Q. africana* were collected from Kribi, South Cameroon, in May 1992 by Paul Mezili. An herbarium specimen is on deposit at the Yaounde National Herbarium.

Extraction and Isolation: Sun-dried and powdered root bark of *Q. africana* (3 kg) was extracted with MeOH (10 l) at room temperature for 5 days. The extract was evaporated *in vacuo* and the resulting dark gum dispersed in H_2O (2 l). The aqueous suspension was extracted successively with Et_2O and EtOAc. Following preliminary bio-assay evaluation for filaricidal activity only the Et_2O extract was further examined. Thus Si gel CC of this extract using cyclohexane-EtOAc (4:1) afforded crude 4-methylthiocanthin-6-one (**1**) which was purified by prep TLC and recrystallization from n-hexane- CHCl_3 mixture (yield 320 mg). Further elution with cyclohexane-EtOAc (1:1) yielded pure 5-methoxycanthin-6-one (300 mg, mp 242-243°), identical in all respects (MS, UV, IR, ^1H NMR) to an authentic sample obtained from *Odyeandyea gabonensis*.
4-Methylthiocanthin-6-one (1): Pale yellow needling, mp 254-255° (Lit [7] 252.5-253.5°); UV λ_{max} (MeOH) nm (log ϵ): 377 (4.15), 361 (4.20), 350 (4.04), 307 (4.10), 296 (4.22), 253 (4.43), 2.36 (4.44); ^1H and ^{13}C NMR spectra: Table 1.; EIMS m/z (rel. int.): $[\text{M}+1]^+$ 267 (26), $[\text{M}]^+$ 266 (100), 237 (35), 221 (15), 205 (10), 193 (7), 164 (12), 139 (12), 113 (17), 112 (15), 103 (20), 84 (35). Found C 67.58, H 3.85, N 10.44, S 12.08. $\text{C}_{10}\text{H}_{15}\text{N}_2\text{O}_5$; requires: C 67.66, H 3.79, N 10.52, S 12.02.

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