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ANTINEOPLASTIC AGENTS 157. *QUASSIA KERSTINGII*[1]

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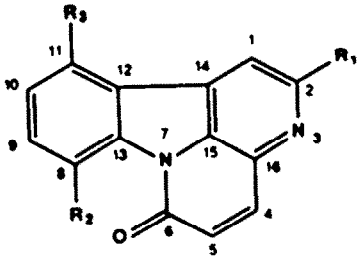
ABSTRACT: The oxacanthin alkaloid components of *Quassia kerstingii* L. have been found to be 2-hydroxy- and 11-hydroxy-canthin-6-one (**1a** and **1b**). Structure **1a** was confirmed by results of methylation (**1a** → **1c** + **2a**) and NOE studies. A selective INEPT NMR technique was employed to support unequivocally the 11-hydroxy-canthin-6-one (**1b**) assignment.

Quassia amora L. and *Q. simaruba* L. (Simaroubaceae) have been employed in primitive medical treatments for cancer.[2] In 1973 *Quassia (Pierrodendron) kertingii* was collected in Ghana and an extract prepared in the U.S. National Cancer Institute's (NCI) programs reached confirmed active status against the P388 lymphocytic leukemia (PS system). By 1975 Kupchan was able to report[3] isolation of the PS active Δ^{13}-dehydroglaucarubinone, a typical Simaroubaceae quassinoid[4-8] from this plant. Subsequently, from part of the same collection of *Q. kerstingii*, we isolated the oxocanthine alkaloids 2-hydroxy-canthin-6-one (**1a**)[9,10] and 11-hydroxy-canthin-6-one (**1b**).[11-13] At that point authentic specimens of these β-carbolines were not available and it became necessary to precisely locate the oxygen substituents.

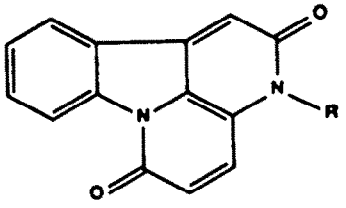
The canthin-6-one nucleus of both β-carbolines was established by ultraviolet, infrared, high field (400 MHz) NMR and mass spectral studies. The hydroxy substituents were located as follows. Methylation of β-carboline **1a** afforded both O- (**1c**) and N-methyl (**2a**) derivatives, a result only consistent with a 2-hydroxy group in equilibrium with the corresponding amide (**2b**). A series of NOE experiments confirmed this conclusion. Irradiation of the methoxy group (**1c**) proton at δ 4.09 produced a 1.5% enchancement of the signal at δ 7.409 (H-1). Saturation of the H-1 singlet effected H-11 (3.5%) and the methoxy group (0.7%, see **3**). Similarly, the H-4 proton was enhanced (4.5%) by irradiating the singlet (at δ 3.799 ppm) exhibited by the N-Methyl proton of amide **2a**(cf., **4**). Thus, the structure was established as 2-hydroxy-canthin-6-one and its tautomer canthin-2,6-dione (**2b**).

Comparison of the physical and spectral data of the second β-carboline (**1b**) isolated with those of 8-hydroxy-canthin-6-one (**1d**)[14] and 11-hydroxy- canthin-6-one[11,13] did not allow a clear choice. So NOE experiments were undertaken (cf. **5**). As no effect on the indole ring protons was noted upon irradiation of H-1, substitution at C-11 seemed likely. Thus selective INEPT NMR experiments were applied to confirm the NOE result.[15] The signals of the proton-bearing carbons were assigned (Fig. 1) on the basis of a ^{1}H-^{13}C two-dimensional chemical shift correlation spectrum. Assignment of the remaining carbon resonances and choice of an 11-hydroxy- or 8-hydroxy-canthin-6-one assignment was made using selective INEPT techniques. The proton-decoupled ^{13}C-NMR spectrum of β-carboline **1b** exhibited seven carbons containing one hydrogen (CH), six fully substituted aromatic carbons and one carbonyl carbon (Fig. 1a). Carbon 12 was expected to show a significant long range coupling with H-1, H-8 (in lieu of a C-11 proton) and H-10. As illustrated in Figs. 1b and 1c, selective irradiation of H-1 (at δ 8.101 ppm) caused polarization transfer to C-12 (111.90 ppm) and C-15 (131.09 ppm). Spectrum 1b (J=7 Hz) uncovered a rather small transfer from H-1 to C-12 and a larger transfer to C-15. The other significant signal present at 146.10 ppm (spectrum 1b) was attributed to the two bond coupling between H-1 and C-2. The effect was more pronounced in spectrum 1c where the polarization transfer was optimized for coupling constants of 4 Hz. The latter spectrum displayed more transfer to C-12 and none to C-15. The two bond coupling constants between ^{1}H and ^{13}C are usually very small, whereas three bond couplings are on the order of 4 to 10 Hz.[16] Generally, the three bond coupling between a proton and a carbon located on two different rings occur at the lower end of this range.

The assignment of C-12 was further substantiated by irradiation of H-8 (δ 7.966) and H-10 (δ 7.017) respectively. Further evidence for the relative proton- carbon positions was obtained by irradiating H-9. Spectral results If and Ig (Fig.1) indicated the transfer from H-9 to C-11 and C-13. Similarly, polarization transfers were observed from H-2 to C-14 and C-16 (Fig. 1h) and H-4 to C-15 and C-6 (Fig. 1i). As no transfers to C-9 occurred in any of these experiments based on using all of the canthine protons, the structure was unequivocally assigned 11-hydroxy-canthin-6-one (1b). Recent comparison with a sample isolated by the Lee group[11] confirmed their mutual identity.



1a: R_1 = OH, R_2 = R_3 = H
1b: R_1 = R_2 = H, R_3 = OH
1c: R_1 = OCH_3, R_2 = R_3 = H
1d: R_1 = R_3 = H, R_2 = OH



2a: R = CH_3
2b: R = H

The selective INEPT technique used herein to verify the structure of canthin 1b should prove useful for other such problems. Interestingly, 11- hydroxy-canthin-6-one (1b) was active against the P388 lymphocytic leukemia cell line showing ED_{50} 2.5 μg/ml whereas the 2-oxo-isomer 1a was found to be inactive in that system.

Table 1. Canthin-6-one ^{1}H-NMR(400 MHz) assignments relative to tetramethylsilane

	Structure No. and solvents					
Position	1a DMSO-d_6	1a DMSO-d_6 + TFA-d (2:1)	1a TFA-d	1b DMSO-d_6	1c $CDCl_3$	2a $CDCl_3$
1	7.407	7.470	7.938	8.101	7.409	7.257
	s	s	s	d,4.88	s	s
2	-	-	-	8.789	-	-
	-	-	-	d, 4.88	-	-
4	7.726	7.721	8.092	8.133	7.880	7.637
	d,9.8	d, 9.56	d, 9.8	d,9.8	d, 9.60	d, 9.8
5	6.845	6.821	7.292	6.978	6.933	6.908
	d, 9.8	d, 9.56	d, 9.8	d, 9.8	d, 9.60	d, 9.8
8	8.464,ddd	8.445	8.611	7.966	8.668	8.644
	7.8,1.2,0.6	d, 8	d, 8.2	d, 8.12	d, 8.24	d, 8.16
9	7.705,dd	7.674,dd	8.104,dd	7.571,dd	7.499,ddd	7.487
	7.8,1.46	(t),8,8	(t),8.2,7.8	8.12,8.04	8.24,8,1.4	m
10	7.579,dd	7.470,dd	7.940,dd	7.017	7.690,ddd	7.683
	7.20,1.2	(t),8,8	7.8,8.2	d, 8.04	8.0,7.7,1.2	m
11	8.287,ddd	8.191	8.284	-	8.061	7.969
	7.20,1.2,0.88	d, 8	d, 8.2		d, 7.70	d, 7.70
OCH_3	-	-	-	-	4.09	-
NCH_3	-	-	-	-	-	3.799

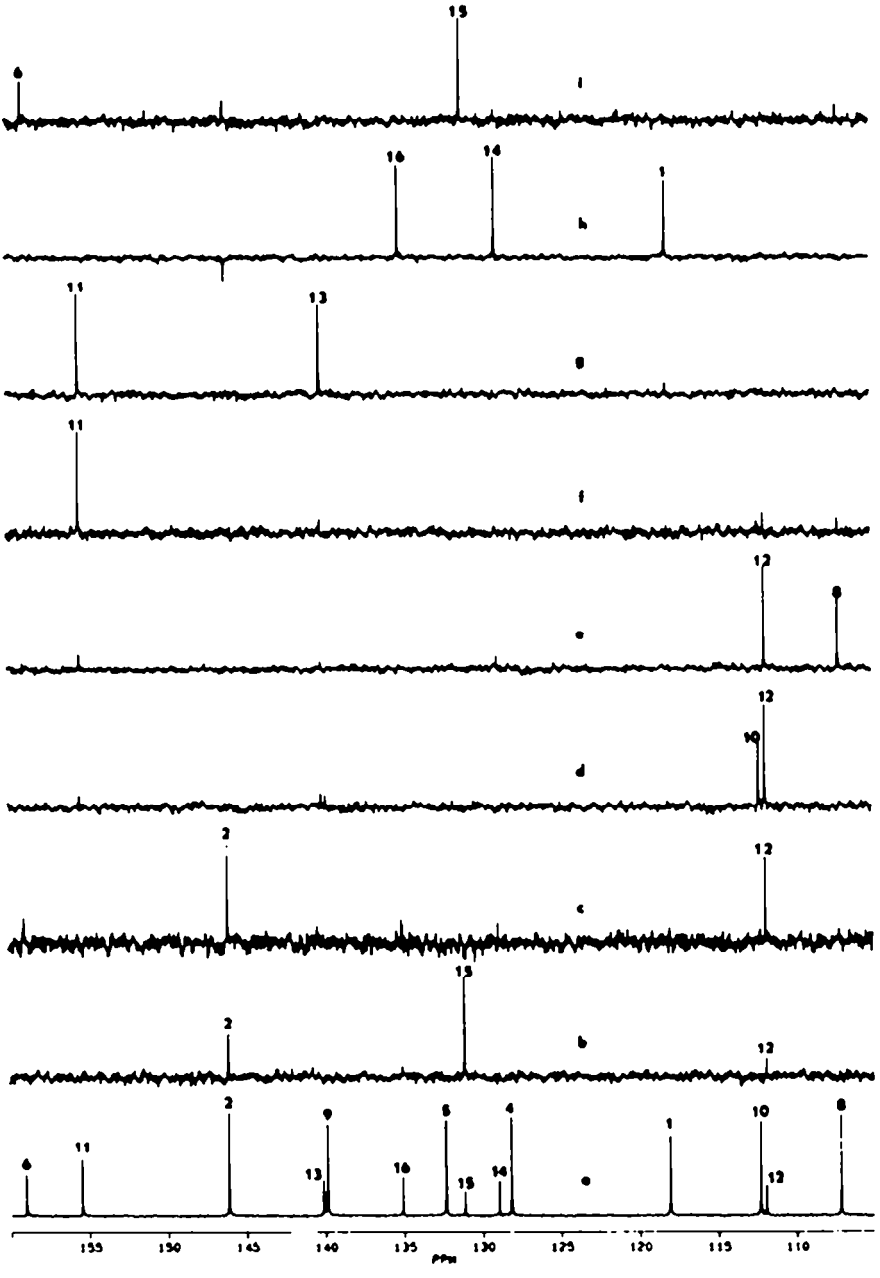
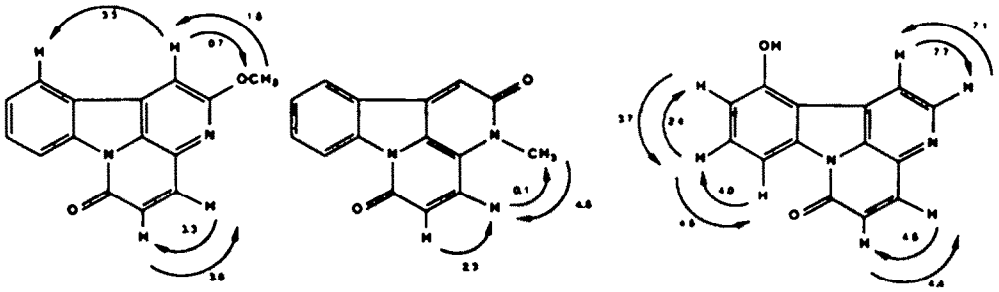


Figure 1: Spectra of 11-hydroxy-canthin-6-one; (a) regular ^{13}C spectrum; (b) - (f) selective INEPT spectra; [(b), (d) - (f) by transfer (J = 7.0 Hz) from H -1, H-8, H-10 and H-9 respectively; (c) and (i) by transfer (J = 4.0 Hz) from H-1 and H-4; (g) and (h) by transfer (J = 10 Hz) from H-9 and H-2].



3 (%NOE) 4 5

Table 2. Canthin-6-one ^{13}C-NMR (100 MHz) assignments relative to tetramethylsilane

	Structure No. and Solvent		
Carbon No.	**1a** TFA-d + D_2O	**1b** DMSO-d_6	**1c** $CDCl_3$
1	108.70 (d)	118.03 (d)	105.23
2	144.44 (s)	146.10 (d)	159.50
4	136.67 (d)	139.83 (d)	138.85
5	126.93 (d)	128.14 (d)	125.59
6	162.57 (s)	159.02 (s)	162.20
8	119.32 (d)	107.15 (d)	117.59
9	132.32 (d)	132.30 (d)	128.23
10	129.59 (d)	112.28 (d)	122.99
11	133.07 (d)	155.44 (s)	131.09
12	124.59 (s)	111.90 (s)	124.51
13	129.23 (s)	140.11 (s)	140.93
14	121.95 (s)	128.91 (s)	131.98
15	125.11 (s)	131.09 (s)	134.10
16	143.56 (s)	135.06 (s)	145.60
OCH_3	-	-	54.40

EXPERIMENTAL

The general experimental and chromatographic procedures have been summarized in reference 17. The NMR spectra were obtained using a Bruker AM- 400 spectroeneter equipped with an Aspect 3000 data system, process controller, and a dual-tuned probehead operating at, respectively, 400.13 MHz and 100.61 MHz for ^1H and ^{13}C. All NOE difference experiments were performed using samples prepared in DMSO-d_6 or $CDCl_3$. Spectra were determined [18] with alternating on- and off-resonance proton irradianon (0.5 to 1.0 s). Selective INEPT experiments were performed according to procedures outlined by Bax.[19,20] using the pulse sequence:

$$^{1}H: 90^{\circ}_{x} - \tau_1 - 180^{\circ}_{x} - \tau_1 - 90^{\circ}_{\pm y} - \tau_2 - 180^{\circ}_{x} - \tau_2 - BB$$
$$^{13}C: \qquad 180^{\circ}_{x} \; - \; 90^{\circ}_{x} \; - \; 180^{\circ}_{x} \; - \; Acq$$

The low-power proton decoupler output was attenuated to provide 90 proton pulses of 13.5 ms at a power of 25 Hz and the decoupler frequency was set to the proton of interest. The delay 1 was set to 62.5, 35.5 or 25.0 ms, which corresponds to ^{1}H- ^{13}C coupling constants of 4.0, 7.0, and 10 Hz. To optimise sensitivity 2 was set to 53.5, 36.5, or 25.0 ms, respectively. The delays 1 and 2 include the length of soft pulses P(90) = 6.45 ms. The two dimensional ^{1}H-^{13}C-shift-correlated spectrum was acquired by the Bax [21] procedure.

PLANT MATERIAL

Stem bark of *Quassia kerstingii* Little (B628279 Simarubaceae family) was collected in Ghana in 1973 as part of the joint NCI-USDA program directed by Dr. J. A. Hartwell.

EXTRACTION AND SOLVENT PARTITIONING

Dried and powdered plant material (6 kg) was extracted (2X) with hot 95% ethanol (32 l) in a Soxhlet apparatus for over 30 hours. The extract was concentrated to 4 l and the solid (inactive, 36.0 g) that separated was collected by filtration. The filtrate was concentrated to a syrupy mass (500 g, PS ED_{50} 4.0 µg/ml, PS *in vivo*, toxic 387 to 48.3 mg/kg). The syrup was partitioned between water (4.5 l) and ethyl acetate (3 x 4.5 l) to afford an inactive fraction (5 g) insoluble in both phases. The bulk (375 g) of the extract remained in the aqueous phase and was inactive (PS). The ethyl acetate fraction was concentrated and further partitioned between methanol- water (4:3, 3 l and hexane (3 x 3 l) to afford an inactive hexane fraction and active aqueous methanol fraction (76 g, PS ED_{50} 1.2 µg/ml, toxic 12.5 to 6.25 mg/kg).

The aqueous methanol fraction (38 g) in methylene chloride was chromatographed on a column of silica gel (1.1 kg, 5 x 132 cm). Elution was begun with methylene chloride and continued with 1% to 5% methanol in methylene chloride. The PS activity was concentrated in two fractions eluted with 5% methanol-methylene chloride: A (1.51 g, ED_{50} 0.25 µg/ml, 39% life extention at 6.25 mg/kg and B (1.42 g, ED_{50} 0.97 µg/ml, 40% life extention at 6.25 mg/kg).

ISOLATION OF 2-HYDROXY -CANTHIN-6-ONE (1a) AND 11-HYDROXY- CANTHIN-6-ONE (1b)

Fraction A was chromatographed on a lobar size C silica gel column and eluted with a gradient of none to 5% methanol in methylene chloride. Continued elution with 5% methanol-methylene chloride afforded a yellow fraction which on crystallization from methanol-methylene chloride yielded yellow needles of 11-hydroxy-canthin-6-one (37.3 mg, 1.24 x 10^{-3}% yield, ED_{50} 2.5 µg/ml): mp 327-9 (lit [10], mp 323-25), UV, IR and mass spectral data were similar to those reported[11,13]. For the ^{1}H-NMR and ^{13}C-NMR data, see Tables 1 and 2.Fraction B was heated in methanol (40 ml) and the solution filtered to give sparingly soluble (methanol) 2-hydroxy-canthin-6-one as a brown powder (16.2 mg, total yield 33.3 mg, 1.1 x 10^{-3}%, PS inactive): mp 388-90 (the brown amorphous solid changes to a fine crystalline state at 265-70), lit [10]. mp 290-305 (dec). The UV, IR and mass spectral data were very similar to that reported[10] except the IR spectrum showed strong absorption at 3437 cm^{-1}, suggesting that crystalline canthine 1a may be predominantly in the hydroxy form. The ^{1}H-NMR and ^{13}C-NMR results have been entered in Tables 1 and 2.

METHYLATION OF 2-HYDROXY-CANTHIN-6-ONE (1a)

A solution of canthine 1b (5 mg) in acetone (5 ml) was heated at reflux (12 h) with anhydrous potassium carbonate (10 mg) and five fold excess methyl iodide. The mixture was cooled to room temperature, potassium carbonate was removed by filtration and the residue concentrated. Two major products were separated on silica-gel plates (hexane-ethyl acetate, 1:1). The bands were eluted with acetone to give O-methyl ether 1c from the upper band as a yellow powder: mp 185-90 ; UV λ_{max} (CH_3OH) 214(4.35), 243(4.16), 253(4.2), 271(3.83), 366(3.72), 385(3.99), 402(3.99) no change was observed in the UV spectrum upon addition of either HCl or NaOMe; IR ν_{max} (NaCl) 1673, 1653, 1640, 1605, 1458, 1433, 1374, 1214, 747 cm^{-1};

HREIMS (m/z) 250.0752 (61.5%, M^+, 250.0815, calcd for $C_{15}H_{10}O_2N_2$), 221.0734 (24%, M^+ +H-CH_2O) and the ^{1}H-NMR and ^{13}C-NMR spectral data are recorded in Tables 1 and 2, respectively. Elution of the lower band furnished amide **2a** as a yellow powder: mp 240-5 , IR ν_{max} (NaCl) 1646, 1616, 1581, 1577, 1560, 1456, 1413 cm^{-1}; HREIMS (m/z) 250.0741 (74%, M^+, 250.0815, calcd for $C_{15}H_{10}O_2N_2$), 221.0733 (22%) and for the ^{1}H-NMR see Table 1.

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