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NEW BIANTHRAQUINONE PIGMENTS FROM *KNIPHOFIA* SPECIES

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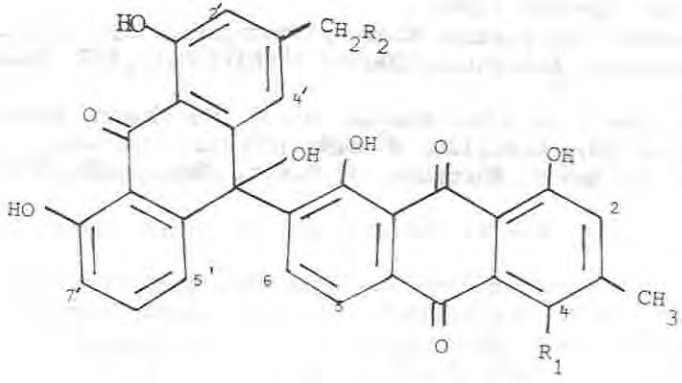
ABSTRACT: Two novel unsymmetrical bianthraquinones, chrysalodin and chryslandicin, were isolated from *Kinphofia* species and their structures dertermined by spectroscopic methods as well as by degradation to known compounds.

INTRODUCTION

We reported recently on the isolation of several anthraquinones from species of *Kniphofia* Moench. (1) and showed the chemotaxonomic significance of anthraquinones to this genus (2). Further investigation of two constituents, earlier designated as Kf2 and Kf8 (2), has now led to the assignment of structures **1** and **2** to these compounds, which shall be named chrysalodin and chryslandicin, respectively. Chrysalodin is restricted to the leaves of *K. foliosa* Hochst., whereas chryslandicin is present in the rhizomes of all species examined (2). Both compounds show borderline cytotoxic activity against in vitro growth of KB tissue culture cells (3).



1. R_1 = H R_2 = OH

2. R_1 = OH R_2 = H

RESULTS AND DISCUSSION

Chrysalodin (1), $C_{30}H_{20}O_9$, forms orange crystals of mp 223-226 °C and shows positive $Mg(OAc)_2$ (4) and alkali tests, indicating a polyhydroxyanthraquinone chromophore. This is corroborated by its UV-visible spectrum with absorption maxima at λ:262, 292, 390 and 435 nm and IR bands at 1680 and 1630 cm^{-1}.

The ^1H NMR data of 1 shown in Table 1 indicated one C-methyl signal at δ 2.44 and a two-proton signal at 4.54 ppm which can be ascribed to a hydroxymethyl group. By spin decoupling experiments it was shown that the methyl group is flanked by protons which resonate at δ 7.08 and 7.56, typical of H-2 and H-4 of an anthraquinone, respectively. Irradiation at the signal of the oxymethylene protons led to a shift in the adjacent H-2' and H-4' protons from a broad singlet at 6.92 to two distinct signals at 6.82 and 6.89, possibly due to energy transfer during irradiation. The protons 5'-H, 6'-H and 7'-H of the anthrone ring give rise to an ABC pattern and the remaining protons 5-H and 6-H appear as an AB quartet at δ 7.96 and 8.79 ppm. The down field shift of the latter signal may be ascribed to the deshielding effect of the neighbouring anthrone system. In DMSO-d_6 the signals for protons 6-H, 4'-H and 5'-H appear strongly broadened which may be explained by the hindered rotation around the C-7-C-10' bond. The hydroxy protons are clearly visible as a triplet at 4.48 and singlets at 6.06, 11.64 (br.), 12.10 (br.), 12.26 and 12.30 ppm.

Table 1. ^1H NMR data of chrysalodin (1) and chryslandicin (2) (400 MHz, acetone-d_6 [ppm]

Proton	1		2	
2-H	7.08	br.s	7.21	br.s
4-H	7.56	br.s	-	
5-H	7.96	d, J = 8 Hz	8.07	d, J = 8 Hz
6-H	8.79	d, J = 8 Hz	8.82	d, J = 8 Hz
2'-H	6.92	br.s	6.75[a]	br.s
4'-H	6.92	br.s	6.76[a]	br.s
5'-H	6.87[a]	d, J = 7.5 Hz	6.87[a]	d, J = 7.5 Hz
6'-H	7.49	dd, J = 7.5/8 Hz	7.48	dd, J = 7.5/8 Hz
7'-H	6.91[a]	d, J = 8 Hz	6.91[a]	d, J = 8 Hz
3-CH_3	2.44	s	2.33	s
3'-CH_2OH	4.54	d, J = 5 Hz[b]	-	
3'-CH_3	-		2.33	s
OH	4.48	t, J =5 Hz,	6.00,	12.22, 12.30[c]
	6.06 s, 11.64,	br., 12.10, br.		
	12.26,	s, 12.30		

[a] Assignments of signals at 6.75/6.76 and 6.87/6.91 ppm may be interchanged. [b] +D_2O: singlet. [c] Further OH signals broadened due to exchange.

The mass spectrum of 1 showed a weak parent peak at m/z 524 and

prominent fragments at m/z 270 and 254 corresponding to ions produced by cleavage of the internuclear bond. Upon reductive cleavage with sodium dithionite in alkaline solution, **1** is quantitatively converted into aloe-emodin and chrysophanol.

The bright red chryslandicin (**2**) earlier designated as Kf8 (2), mp 250°C(dec.), revealed a parent ion at m/z 524 in the MS corresponding to $C_{30}H_{20}O_9$. The pigment showed positive anthraquinone tests and the presence of carbonyl bands at 1645 and 1600 cm^{-1} in the IR spectrum strongly suggested the presence of a 1,4-dihydroxyanthraquinone moiety (5). This hydroxylation pattern is further supported by the purple colour produced by the addition of 2 to an alcoholic solution of magnesium acetate (4). The ^1H NMR spectrum of 2 showed two C-methyl signals at 2.23 and 2.33 ppm and further signals in the aromatic region which are in accord with formula **2**. The assignments were confirmed by decoupling experiments. Further proof of this structure was obtained by cleavage with alkaline sodium dithionite which afforded chrysophanol and islandicin. The cleavage products were identified by spectroscopic means as well as by comparison with authentic samples. Interestingly both these monomers co-exist in the rhizomes with chryslandicin (**2**) which indicates a close biogenetic relationship.

Chrysalodin (**1**) and chryslandicin (**2**) have a similar general structure to the bianthraquinone (pigment II) isolated from *Aloe saponaria* by Yagi and co-workers (6).

EXPERIMENTAL

Isolation of anthraquinones: For general details of the collection of *Kniphofia* Spp. and for the extraction and separation of the anthraquinones see also ref. (1) and (2). Chrysalodin (**1**) was obtained from the acetone extract of the leaves of *K. foliosa* collected in Bale region, Ethiopia, in September 1985. The crude extract, upon TLC examination, using the solvent system C_6H_6/EtOAc/dichloromethane 7:1:1, was shown to contain in addition to compound **1** (R_f 0.51) other anthraquinones, particularly aloe-emodin (R_f 0.56), kinpholone (R_f 0.62), aloe-emodin acetate (R_f 0.78), and chrysophanol (R_f 0.79) (7). Column chromatography on silica gel 60 (Merck), using gradient elution with petroleum ether/acetone followed by preparative TLC afforded pure chrysalodin (**1**). The isolation of chryslandicin (**2**) was effected according to the method described in ref. (1).

Chrysalodin (**1**): Orange crystals (benzene), mp 223-228°C; IR υ_{max} (KBr) cm^{-1}: 3500, 1670, 1630, 1460, 1450, 1280; UV λ_{max} (MeOH) nm: 435, 390, 292, 262, 228; CD (MeOH): 435^{-3270}, 330^{0}, 308^{+3630}, 295^{+2820}, 280^{0}, 268^{-15280}, 231^{-34550} (last reading); MS (70 eV): m/z (rel. int. %) 524.1110 (0.6, M^+; calc. for $C_{30}H_{20}O_9$ 524.1107), 506 (1.7, $C_{30}H_{18}O_8$), 476 (2.6, $C_{29}H_{16}O_7$), 270 (10.7, $C_{15}H_{10}O_5$), 254 (10.6, $C_{15}H_{10}O_4$); ^1H NMR see Table 1.

Chryslandicin (**2**): Bright red crystals (benzene/methanol), mp 250°C(dec.), $[\alpha]_D$+107 (MeOH; c 0.008); IR υ_{max} (KBr) cm^{-1}: 3500, 2930, 1645, 1615, 1460, 1280 UV λ_{max} (MeOH) nm: 500, 386, 297, 256, 233; MS (70 eV):m/z (rel. int. %) 524.1105 (5.8 M^+; calc. for

$C_{30}H_{20}O_9$ 524.1107), 506(100,$C_{30}H_{18}O_8$), 270, 254; ^1H NMR see Table 1.

Reductive cleavage of compounds 1 and 2: Both compounds were reductively cleaved using the method described in ref. (2). The cleavage products chrysophanol and aloe-emodin from compound 1 and chrysophanol and islandicin from compound 2 were characterized both by spectroscopic methods as well as by comparison with authentic samples.

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