Pergamon 0031-9422(95)00461-0 *Phytochemistry*, Vol. 40, No. 5, pp. 1429-1431, 1995

0031-9422/95 $9.50 + 0.00

ANTHRACENONES AND ANTHRAQUINONES FROM ROOTS OF FIVE SPECIES OF THE GENUS *KARWINSKIA*

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(*Received in revised form* 8 *May* 1995)

Key Word Index—*Karwinskia umbellata*; *K. subcordata*; *K. humboldtiana*; *K. mollis*; *K. johnstonii*; Rhamnaceae; anthracenone; anthraquinone; neurotoxin; ^{13}C NMR.

Abstract—A new polyphenolic anthracenone has been isolated from the roots of *K. umbellata*, *K. subcordata*, *K. humboldtiana*, *K. mollis* and *K. johnstonii* together with three known structural analogues. The new compound has been characterized as 7-[3′,4′-dihydro-9′-methoxy-1′,3′-dimethyl-10′-hydroxy-1*H*′-naphtho[2′,3′,*c*′]pyran-5′-yl]-3,4-dihydro-3-methyl-3,8,9-trihydroxy-1(2*H*)-anthracenone. The chemical shifts of the ^{13}C NMR spectra of this anthracenone and a related anthraquinone are reported.

INTRODUCTION

The fruits of the neurotoxic plants of the genus *Karwinskia* (Rhamnaceae) have been extensively studied chemically and toxicologically [1–5], and the presence of the neurotoxin **1** and anthraquinones **3** and **4** from the fruit of *K. humboldtiana* has been described [6, 7]. The previous isolation [6] of **3** strongly suggested the presence of its probable biogenetic precursor **2**. However, repeated attempts to detect it chromatographically, in the original extract of the fruit, were unsuccessful. On the other hand, there are no chemical studies of the roots of this important genus, except for *K. humboldtiana*, where compound **1**, eleutergonzalone, the flavones baicalein and quercetin [8] and more recently karwinaphthols A and B [9] were isolated. In addition, in some regions of Mexico, the aqueous extracts from the fresh root of some toxic species of this genus are administered against the paralytic effect of neurotoxin **1**, when the latter has recently been eaten with the fruit [10]. Consequently, these antecedents prompted us to extend the chemical studies to the roots of other species of the title genus.

RESULTS AND DISCUSSION

The hexane extracts of the dried powdered roots of *K. umbellata*, *K. subcordata*, *K. humboldtiana*, *K. Johnstonii* and *K. mollis* were chromatographed on silica gel, affording anthraquinones **3** and **4** as yellow powders, which were identified by comparison of the ^{1}H NMR and physical data with those reported in the literature [6, 7]. A second methylene chloride extraction provided, after repeated column chromatography on silica gel, the neurotoxin **1** and the new anthracenone **2**. The latter showed an UV absorption maximum at 237 nm similar to that of **1**, and its IR spectrum showed a strong absorption band at 3389 cm^{-1}, due to hydroxyl groups, and a chelated carbonyl group absorption at 1626 cm^{-1}.

The ^{1}H NMR spectrum of **2** showed the presence of a single methoxyl group signal at δ4.06, indicating that this group is at the C-9′ position, in agreement with the corresponding data for **1** and **3** [6]. Therefore, the aromatic region for this moiety had two pairs of double doublet signals (*ortho* and *meta* couplings) at δ6.98 (*dd*, $J = 8.1$ and 0.9 Hz, H-6′) and 6.73 (*dd*, $J = 8.1$ and 0.9 Hz, H-8′) and a triplet signal at δ7.13 (app *t*, $J = 8.1$ Hz, H-7′). As described for similar compounds [7], the presence of the corresponding atropisomers of **2** was detected by observing the doubling of the resonance signals for H-7′ (δ7.13 and 7.12), H-6′ (δ6.98 and 6.94) and Me-3′ (δ1.22 and 1.23); it was also observed that the ratio of the atropisomers of **2** is different for each species. The remaining signals were essentially identical to **1** [6]. In addition, the relative stereochemistry of C-1′ and C-3′ of the dihydrodimethylpyran ring was determined by a NOE difference experiment, giving a correlation between H-1′ and H-3′ (6.4%, enhancement of H-1′), proving that both protons are pseudoaxial as assumed for **1** [7]. In order to obtain additional structural evidence, oxidation of **2** in air gave anthraquinone **3**, whose ^{1}H NMR data were identical with those reported [6].

With respect to the assignment of the ^{13}C NMR spectra of **2** and **3**, the great number of quaternary

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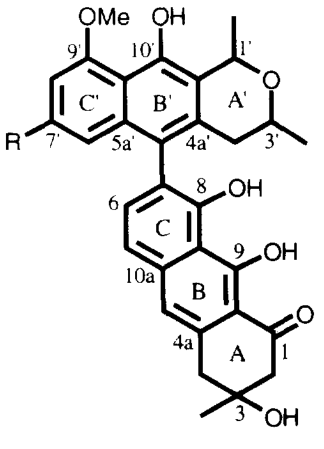
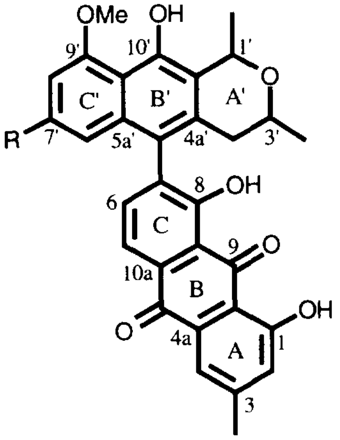


1 R = OMe
2 R = H



3 R = H
4 R = OMe

aromatic carbons makes it difficult to assign them. However, all the assignments were attributed on the basis of the one-bond and long-distance heteronuclear couplings obtained by using GATED decoupling and 2D heteronuclear correlation (HETCOR and long-range heteronuclear shift correlation). From Table 1, it can be observed that the chemical shifts of the naphthopyran system (A', B' and C' rings) are consistent for both compounds, while the chemical shifts for the anthracenone and anthraquinone moieties (A, B and C rings) are in accordance with those of related structures of torosachrysone [11] and chrysophanol [12], respectively. It should be noted that the frequencies of the C-10a and C-5a' on anthraquinone **3** are very close, and the signals of CH_3-1' and CH_3-3' have the same frequency. Additional signals at 134.1 and 35.7 ppm could be attributed to C-4a' and C-4', respectively, for the minor atropisomer as expected. The assignments of the ^{13}C NMR spectra of these compounds could be useful to determine those of structurally related naturally occurring compounds [6], as well as those of semi-synthetic compounds [7]. On the other hand, the analysis by ^{1}H NMR in the methoxyl group region (δ4.06 MeO-9' in **2**, 4.01 MeO-9' and 3.57 MeO-7' in **1**) of the crude mixtures, obtained by precipitation of the methylene chloride extracts with cold hexane, allowed us to determine the approximate relative proportion of **1** and **2** present in these five species (Table 2). These results could be of chemotaxonomic significance for the classification of this genus.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were obtained at 300 and 75.4 MHz, respectively, using $CDCl_3$ as solvent and TMS as int. standard. Mps: uncorr.

Table 1. ^{13}C NMR chemical shifts* (δ) of the anthracenone **2** and anthraquinone **3**

Carbon	**2** δ(ppm)	Carbon	**3** δ(ppm)
1	203.3	1	162.9
2	51.1	2	124.5
3	70.7	3	149.5
4	43.1	4	121.5
4a	134.9	4a	133.5
5	118.4	5	120.0
6	136.3	6	140.3
7	121.1	7	136.0
8	154.8	8	160.4
8a	112.8	8a	116.1
9	165.5	9	192.9
9a	109.5	9a	114.0
10	118.5	10	182.1
10a	139.1	10a	133.25†
Me-3	28.8	Me-3	22.4
1'	71.2	1'	71.3
3'	69.4	3'	69.4
4'	36.6	4'	36.9
4a'	134.4	4a'	134.5
5'	124.0	5'	122.1
5a'	133.6	5a'	133.22†
6'	119.6	6'	119.2
7'	125.1	7'	125.7
8'	103.4	8'	103.8
9'	156.2	9'	156.5
9a'	113.4	9a'	113.6
10'	150.0	10'	151.0
10a'	121.4	10a'	121.7
Me-1'	21.7	Me-1'	21.7
Me-3'	21.6	Me-3'	21.7
OMe	56.1	OMe	57.1

*Measured in $CDCl_3$.
†These signals may be reversed.

Table 2. Ratio of compounds **1** and **2** of the *Karwinskia* species

Species	**1**:**2**
K. humboldtiana	33:67
K. umbellata	17:83
K. subcordata	1:1
K. mollis	3:22
K. johnstonii	3:7

Plant materials. The species were collected in Mexico as follows: *K. subcordata*, *K. humboldtiana* and *K. mollis* (October–November, 1992) from Ixmiquilpan, Hidalgo State; *K. umbellata* (October, 1992) from Puente de Ixtla, Morelos State; *K. johnstonii* (February, 1994) from Infiernillo, Michoacán State. Voucher specimens were collected and deposited in the herbarium of the Escuela Nacional de Ciencias Biológicas, Department of Botany, México City, by Dr Rafael Fernández Nava.

Isolation. For all the species, the following general procedure was carried out. The dry powdered root (400 g) was extracted with hexane (1 l) for 2 days at room temp. The residue was subjected to CC on silica gel (hexane–EtOAc, 9:1) to furnish **3** and **4**, the latter in minor quantity from *K. umbellata* and *K. mollis*. The root was extracted with CH_2Cl_2 (1 l) for 3 days at room temp. and the partially evap extract was treated with cold hexane to obtain a crude ppt. formed principally of a mixt. of **1** and **2**, which was analysed by ^1H NMR. This ppt. was subjected to repeated CC on silica gel (hexane–EtOAc, 1:1) to give pure **1** and **2**. Compounds **1**, **3** and **4** were identified by comparing physical and spectral data previously described [6, 7].

Compound **2**. The following data correspond to the atropisomers mixt. of **2** isolated from *K. umbellata*. Yellow powder, mp 168–169°. UV λ_{max} (EtOH) nm (log ε): 237 (4.74), 271 (4.52), 309 (4.04), 415 (3.99), IR ν_{max} cm^{-1} ($CHCl_3$): 3389, 1626, 1606, 1418, 1380, 1331, 1244, 1071. ^1H NMR ($CDCl_3$) major atrop. [minor atrop.]: δ16.03 (*s*, 1H, HO-9), 9.86 (*s*, 1H, HO-10′ or HO-8), 9.85 (*s*, 1H, HO-10′ or HO-8), 7.41 (*d*, 1H, J = 8.2 Hz, H-6), 7.30 (*d*, 1H, J = 8.2 Hz, H-5), 7.13 [7.12] (*t*, J = 8.1 Hz, H-7′), 7.12 (*s*, 1H, H-10), 6.98 [6.94] (*dd*, J = 8.1, 0.9 Hz, H-6′), 6.73 (*dd*, J = 8.1, J = 0.9 Hz, H-8′), 5.29 (*q*, 1H, J = 6.3 Hz, H-1′), 4.06 (*s*, 3H, MeO), 3.71 (*m*, 1H, H-3′), 3.15 (AB system, 2H, H-4a and H-4b), 2.89 (AB system, 2H, H-2a and H-2b), 2.38 (*m*, 2H, H-4a′ and H-4b′), 1.70 (*d*, 3H, J = 6.3 Hz, Me-1′), 1.50 (*s*, 3H, Me-3), 1.22 [1.23] (*d*, 3H, J = 6.1 Hz, Me-3′). ^{13}C NMR: see Table 1 for the major atropisomer. Signals attributed to the minor atropisomer: 134.1 (C-4a′) and 35.7 (C-4′).

Oxidation of **2**. A soln of 100 mg (0.19 mmol) of **2** in 5 ml pyridine was stirred for 2 h while a stream of air was passed through the soln. The pyridine was removed and the residue was extracted with EtOAc, washed with 10% aq. HCl, dried and chromatographed on silica gel, giving 35 mg (36%) of **3**.

Acknowledgement—We thank DEPI-IPN (Grants: 932906 and 932914) for partial financial support.

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