Phytochemistry, Vol. 24, No. 8, pp. 1681–1683, 1985
Printed in Great Britain

0031–9422/85 \$3.00+0.00
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ANTIMICROBIAL AGENTS FROM HIGHER PLANTS: TWO DIMETHYLBENZISOCHROMANS FROM *KARWINSKIA HUMBOLDTIANA*

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(*Received* 6 *November* 1984)

Key Word Index—*Karwinskia humboldtiana*; Rhamnaceae; karwinaphthol A and B; 2-acetyl-6,8-dimethoxy-3-methyl-1-naphthol; eleutherin and 7-methoxyeleutherin; antimicrobial activity.

Abstract—Two new antimicrobial dimethylbenzisochromans were isolated from the roots of *Karwinskia humboldtiana* together with the known 7-acetyl-6,8-dimethoxy-3-methyl-1-naphthol. The structures and absolute configurations were determined by spectroscopic examination and by chemical transformation to the known quinones eleutherin and 7-methoxyeleutherin.

INTRODUCTION

Karwinskia humboldtiana (Rhamnaceae) is a poisonous plant growing abundantly in southwest Texas, lower California, Mexico, and parts of Central America. The fruit pulp is edible, but the seeds are poisonous. They are used locally in Mexico to treat convulsions [1]. Previous reports describe the isolation and identification of several cytotoxic and nontoxic constituents from the seeds [2–4] and roots [5] as well as a partially characterized cytotoxic principle [6]. We became interested in the plant when extracts of the roots showed reproducible activity *in vitro* in an agar dilution assay against *Staphylococcus aureus* and *Mycobacterium smegmatis* at the 1000 and $< 100\,\mu g/ml$ level [7]. Fractionation was therefore undertaken [8].

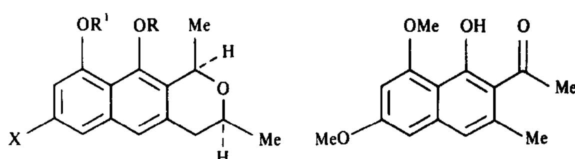
RESULTS AND DISCUSSION

The air-dried, ground roots of *K. humboldtiana* were exhaustively extracted with dichloromethane and then 95% ethanol in a Soxhlet apparatus. Silica gel chromatography produced three active constituents: karwinaphthol A (**1**), karwinaphthol B (**2**) and 2-acetyl-6,8-dimethoxy-3-methyl-1-naphthol (**3**).

Karwinaphthol A (**1**) has an empirical formula of $C_{16}H_{18}O_3$. The UV absorption was characteristic of an 8-methoxy-1-naphthol [9]. ^1H NMR doublets at δ1.35 and 1.65 are assigned to two secondary methyls attached to an oxygen-bearing carbon. The farthest downfield, presumably benzylic, of these can be associated with a quartet at δ5.16. The other methyl group is associated with a methine multiplet centred at δ3.70. This signal is further associated with a remaining aliphatic methylene multiplet at δ2.70. These signals are consistent with the presence of a 1,3-dimethylpyran moiety in karwinaphthol A. A methoxyl signal was visible at δ4.01 as were four aromatic hydrogen signals consisting of a singlet at δ6.93 and an ABC multiplet. The spectrum was rounded out by an exchangeable phenolic hydroxyl signal at δ9.59. These data suggested structures **1** and **1a** as most likely.

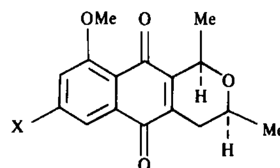
The structure was proven conclusively to be **1** by oxidation with Fremy's salt to the known quinone, eleutherin (**4**). The stereochemistry of the methyl groups follows from the work of Cameron *et al.* [10] who established them to be *cis* and from the optical rotations which demonstrated absolute configurational identity, as expected. While **1** has not been encountered previously in nature, it has been prepared in racemic form by Webb *et al.* in the course of a biogenetically patterned synthesis of eleutherin [11].

Karwinaphthol B (**3**) has an empirical formula of $C_{17}H_{20}O_4$. The ^1H NMR signals at 1.34, 1.63 along with a methine quartet at δ5.14 and a two proton multiplet at 2.72 plus an obscured methine multiplet at δ3.90 are very similar to those seen for karwinaphthol A and suggest a 1,3-dimethylpyran ring as a part of the structure of karwinaphthol B also. Two methoxyl singlets at δ3.84 and 3.97 are sufficient to account for the difference in mass



1 R = H R' = Me X = H
1a R = Me R' = H X = H
2 R = H, R' = Me X = OMe
2a R = Me, R' = H, X = OMe

3



4 X = H
5 X = OMe

Table 1. Antimicrobial activity of *Karwinskia humboldtiana* constituents

Compound	Microorganism* (μg/ml)						
	1	2	3	4	5	6	7
Karwinaphthol (**1**)	i	i	i	i	12.5	i	i
Karwinaphthol (**2**)	i	i	i	i	50	i	i
2-Acetyl-6,8-dimethoxy-3-methyl-1-naphthol (**5**)	i	i	i	i	100	i	i
Eleutherin (**3**)	50	i	i	i	100	i	i
7-Methoxyeleutherin (**4**)	100	i	i	i	100	i	i
Streptomycin sulphate	5	5	5	2.5	1.25	i	i

*Microorganism 1, *Staphylococcus aureus* ATCC 13709; 2, *Escherichia coli* ATCC 9637; 3, *Salmonella gallinarum* ATCC 9184; 4, *Klebsiella pneumoniae* ATCC 10031; 5, *Mycobacterium smegmatis* ATCC 607; 6, *Candida albicans* ATCC 10231; 7, *Pseudomonas aeruginosa* ATCC 27853. Crude extracts were tested by agar-dilution streak methods at 1000 and 100 μg/ml. Pure compounds were tested in the same manner starting at 100 μg/ml and diluting by a factor of 2 until an endpoint was reached. The numbers refer to the last concentrations at which no visible growth occured. Those substances or preparations which did not inhibit at the highest level tested are listed as inactive (i).

between karwinaphthols A and B. The hydroxyl showed IR absorption at 3500 cm^{-1} and an ^1H NMR signal at δ9.37. The three aromatic protons appeared at 6.63, 6.59 (*d*, $J = 2$ Hz) and 6.69 (*s*). Thus the additional methoxyl must be in the left hand aromatic ring and is *meta* disposed to the other methoxyl. A choice between the two possible structures for karwinaphthol B (**2** and **2a**) was readily made in favour of **2** by oxidation of karwinaphthol B to 7-methoxyeleutherin [2] (**5**) with Fremy's salt.

Column chromatography of fractions 9–14 gave a crystalline compound shown by its properties to be identical with 2-acetyl-6,8-dimethoxy-3-methyl-1-naphthol (**3**) previously isolated from the seeds of this plant [2, 12].

The antimicrobial activity of these compounds is set forth in Table 1 from which it can be seen that except for karwinaphthol the potency is weak and the spectrum is narrow. Despite the ease with which oxidation of the karwinaphthols to the quinones takes place, the karwinaphthols are significantly more active. Thus intrinsic activity of the karwinaphthols is indicated. Our present efforts centre around the identification of the antimicrobial agents present in the alcoholic extracts. Preliminary chromatographic evidence has established that these are not identical to the products described herein.

It is interesting to note that although *Karwinskia humboldtiana* has been the subject of several previous studies, the application of fractionation techniques in conjunction with bioassays was required to reveal the presence of these two new natural compounds.

EXPERIMENTAL

Plant material. *Karwinskia humboldtiana* was collected by Professor Michael Powell, Department of Biology, Sul Ross State University, Alpine, Texas, in the fall of 1983 on the Boca Chica road near Brownsville, Cameron Country, Texas.

Extraction. Dried and powdered roots (450 g) of *Karwinskia humboldtiana* were extracted with CH_2Cl_2 and 95% EtOH for 48 hr successively. The CH_2Cl_2 extract was concd under red. pres. at 40° to give a dark reddish-brown antimicrobially-active residue (5.6 g). The residue (5 g) was dissolved in CH_2Cl_2 (5 ml) and applied to a silica gel column (50 g) packed in CH_2Cl_2. Elution with CH_2Cl_2, CH_2Cl_2–MeOH (19:1) and CH_2Cl_2–MeOH (9:1) yielded a number of fractions which were combined based upon TLC monitoring. Fractions of 10 ml were collected.

Isolation of karwinaphthol A. The semicrystalline residue from fractions 14 and 15, on crystallization from Et_2O–hexane gave needles of karwinaphthol A (35 mg); mp 133.5–134°; $[\alpha]_D^{24}$ +187° (*c* 0.842; $CHCl_3$); CD $[\theta]_{max}^{MeOH}$ nm: 235 (+12 900), 245 (−6500), 305 (+5550), 318 (+5020), 335 (+5085); UV λ_{max}^{MeOH} nm (log ε): 241 (4.74), 270 (3.38), 275 (3.49), 282 (3.49), 289 (3.61); $\lambda_{max}^{MeOH-HCl}$ nm (log ε): 242 (4.76), 270 (3.72), 276 (3.87), 284 (3.87), 291 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3500, 3000, 2980, 2830, 1640, 1620, 1590, 1510, 1450, 1430, 1370 (sh), 1360, 1330, 1290, 1220, 1160, 1080, 1010, 960, 830, 750; ^1H NMR ($CDCl_3$): δ1.35 (3H, *d*, *J* = 6 Hz), 1.65 (3H, *d*, *J* = 6 Hz), 2.70 (2H, *m*), 3.70 (1H, *m*), 4.01 (3H, *s*), 5.16 (1H, *q*, *J* = 6 Hz), 6.63 (1H, *dd*, *J* = 2.5, 6.2 Hz), 6.93 (1H, *s*), 7.22 (2H, *m*), 9.59 (1H, *s*); MS *m/z* (rel. int.): 258.12436 (calc. for $C_{16}H_{18}O_3$ 258.12549) (26%), 244 (18), 243 (100), 228 (28), 225 (7), 214 (6), 171 (5), 152 (8), 128 (8), 115 (8), 77 (6), 43 (14).

Isolation of karwinaphthol B (**2**). Fractions 16–18 gave a light yellow residue (60 mg) after removal of solvent. This was redissolved in C_6H_6–hexane (1:1, 2 ml) and applied to a silica gel column (10 g) set with C_6H_6–hexane (1:1) and eluted with the same solvent. Fractions 15–18, containing pure compound, were mixed and the solvent was removed to obtain a light yellow residue (35 mg) which resisted crystallization from several solvent systems. TLC of the residue with several solvent systems demonstrated homogeneity. $[\alpha]_D^{24}$ +141° (*c* 0.754; $CHCl_3$); CD $[\theta]_{max}^{MeOH}$ nm: 243 (30 195), 255 (−8050), 290 (4650), 302 (5030), 323 (2010), 335 (2010); UV λ_{max}^{MeOH} nm (log ε): 243 (4.65), 257 (3.72), 262 (3.74), 268 (3.76), 275 (3.74), 286 (3.61), 293 (3.62), 316 (3.27); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3000, 2950, 2875, 2250, 1610, 1590, 1450, 1400, 1380, 1310, 1260, 1200, 1180, 1170 (sh), 1100, 1050, 1010, 990, 920 (br), 860, 820; ^1H NMR ($CDCl_3$): δ1.34 (3H, *d*, *J* = 6 Hz), 1.63 (3H, *d*, *J* = 6 Hz), 2.72 (2H, *m*), 3.70 (1H, *m*, obscured), 3.84 (3H, *s*), 3.97 (3H, *s*), 5.14 (1H, *q*, *J* = 6 Hz), 6.36 (1H, *d*, *J* = 2 Hz), 6.59 (1H, *d*, *J* = 2 Hz), 6.89 (1H, *br s*), 9.37 (1H,

s); MS *m/z* (rel. int.): 288.13531 (calc. for $C_{17}H_{20}O_4$, 288.13604) (29 %), 273 (100), 243 (9), 229 (7), 141 (5), 139 (6), 129 (9), 115 (16), 43 (32).

Isolation of 2-acetyl-6,8-dimethoxy-3-methyl-1-naphthol (**3**). The semicrystalline residue from fractions 9–14, on crystallization from Et_2O–hexane, gave needles of 2-acetyl-6,8-dimethoxy-3-methyl-1-naphthol (30 mg): mp 99–99.5° (lit. [12] mp 98–99°; ^1H NMR ($CDCl_3$): δ2.34 (3H, *s*), 2.60 (3H, *s*), 3.87 (3H, *s*), 4.01 (3H, *s*), 6.42 (1H, *d*, J = 2 Hz), 6.50 (1H, *d*, J = 2 Hz), 6.95 (1H, *s*), 9.73 (1H, *s*); MS *m/z* (rel. int.): 260 (52 %), 245 (100), 229 (3), 201 (6), 131 (6), 115 (12), 102 (6), 77 (9), 43 (23).

Oxidation of karwinaphthol A (**1**) *to eleutherin* (**4**). Karwinaphthol A (15 mg) was dissolved in DMF and added to an aqueous soln of $(KSO_3)_2NO$ (60 mg) and KH_2PO_4 (35 mg), stirred for 10 min at 5° under N_2, acidified with dil. HCl and extracted with EtOAc. The EtOAc layer was washed with H_2O, dried and evaporated to give a yellow residue (12 mg). The residue was dissolved in C_6H_6 (0.5 ml) and applied to a silica gel column (10 g) set with C_6H_6–EtOAc (19.1). Fractions 9–12, containing a yellow band, were mixed and the solvent was removed under vacuum. The residue was crystallized from CH_2Cl_2–hexane to give yellow needles (10 mg): mp 174–175° (lit. [13] mp 175°); $[\alpha]_D^{26}$ +345 (*c* 0.433; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2950, 2870, 1660, 1600, 1560, 1460, 1430, 1350, 1325, 1280, 1220, 1200, 1160, 1070, 1060, 1000, 860, 830; ^1H NMR ($CDCl_3$): δ1.35 (3H, *d*, J = 6 Hz), 1.53 (3H, *d*, J = 6 Hz), 2.20 (1H, *ddd*, J = 3.7, 8, and 18 Hz), 2.85 (1H, *dt*, J = 1.4, 4, and 18 Hz), 3.60 (1H, *m*), 3.98 (3H, *s*), 4.82 (1H, *m*), 7.27 (1H, *m*), 7.65 (2H, *m*); MS *m/z* (rel. int.): 272.10466 (calc. for $C_{16}H_{16}O_4$, 272.10476) (37 %), 257 (59), 243 (34), 239 (20), 229 (11), 214 (13), 157 (8), 135 (11), 128 (17), 115 (19), 92 (11), 76 (36), 43 (100). A sample cochromatographed (silica gel, hexane–Et_2O, 7:3, R_f 0.20) with an authentic sample of eleutherin and separated from an authentic sample of isoeleutherin (silica gel, C_6H_6–EtOAc, 9:1, eleutherin R_f 0.46, isoeleutherin R_f 0.38).

Oxidation of karwinaphthol B (**2**) *to 7-methoxyeleutherin* (**5**). Karwinaphthol B (15 mg) in DMF (1 ml) was added to an aq. soln of $(KSO_3)_2NO$ (60 mg) and KH_2PO_4 (35 mg) and stirred for 10 min under N_2, acidified with dil. HCl and extracted with EtOAc (5 ml). The EtOAc layer was washed well with H_2O, dried and the solvent removed under red. pres. The residue was dissolved in C_6H_6 and applied to a silica gel column (10 g) set with C_6H_6–EtOAc (9:1). Fractions 5–7 containing a yellow band were combined and concd to give a yellow residue. The residue was crystallized from CH_2Cl_2–hexane to give yellow needles of 7-methoxyeleutherin (7 mg): mp 153–154°; $[\alpha]_D^{24}$ +265° ($CDCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3000, 2950, 2875, 1660, 1600, 1560, 1440, 1420, 1350, 1320, 1280, 1220, 1200, 1160, 1070, 1060 (sh), 1000, 850, 830; ^1H NMR ($CDCl_3$): δ1.35 (3H, *d*, J = 7 Hz), 1.53 (3H, *d*, J = 7 Hz), 2.15 (1H, *ddd*, J = 3.7, 9, and 18 Hz), 2.75 (1H, *dt*, J = 1.5, 3, and 18 Hz), 3.55 (1H, *m*), 3.93 (3H, *s*), 3.95 (3H, *s*), 4.83 (1H, *m*), 6.67 (1H, *d*, J = 2.5 Hz), 7.20 (1H, *d*, J = 2.5 Hz); MS *m/z* (rel. int.): 302.11432 (calc. for $C_{17}H_{18}O_5$, 302.11531) (48 %), 287 (53), 273 (76), 255 (8), 244 (13), 243 (13), 229 (13), 217 (10), 215 (11), 201 (8), 151 (18), 128 (11), 115 (18), 106 (26), 77 (23), 69 (24), 63 (38).

Acknowledgements—The authors thank the National Institutes of Health (U.S.A.) for partial support of this work under grant AI-13155; Professor Michael Powell, Department of Biology, Sul Ross State University, Alpine, Texas for providing plant material; Professor Albert Burgstahler, Department of Chemistry, University of Kansas, Lawrence, Kansas, for his assistance with the circular dichroism measurements; Professor Constance Harris of Vanderbilt University for an authentic (synthetic) sample of eleutherin; Dr. Hajime Komura of the SUNBOR Institute, Osaka, Japan, and Professor Ren-sheng Xu of the Shanghai Institute of Materia Medica, Shanghai, People's Republic of China, for an authentic sample of isoeleutherin.

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