

FLAVONOIDS FROM THE EXUDATE OF *ACACIA NEOVERNICOSA*

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Key Word Index—*Acacia neovernicosa*; Leguminosae; Mimosoideae; 2',4' - dihydroxychalcone; 4' - hydroxy - 2' - methoxychalcone; 2',4' - dihydroxy - 3' - methoxychalcone; 2',4',4 - trihydroxychalcone; 7 - hydroxyflavanone; pinocembrin.

Abstract—2',4'-Dihydroxychalcone, 4'-hydroxy-2'-methoxychalcone and 2',4'-dihydroxy-3'-methoxychalcone were isolated and characterized from the resinous exudate produced by *Acacia neovernicosa*. Smaller amounts of isoliquiritigenin, pinocembrin and chrysin were also found and identified by their chromatographic properties and UV spectra. The material of one collection contained galangin, 3-methylkaempferol and 3,3' - dimethylquercetin.

INTRODUCTION

Acacia neovernicosa Isely is a small spiny shrub which is widespread throughout the south-western United States and northern Mexico. The outer surface of the leaves and young stems is viscid-vernicose and covered with minute glands. The resinous material is readily removed by non-polar solvents such as chloroform and is similar in properties to several others recently described [1]. In this report we describe the identification of flavonoids which are components of the 'resin' of *A. neovernicosa*.

RESULTS AND DISCUSSION

The materials on the outside of *A. neovernicosa* leaves and stems were isolated by washing with chloroform. The major portion of these 'resinous' materials was non-polar lipids which were removed by CC. These materials were usually eluted at or near the solvent front. Another significant portion of the resinous materials is comprised of flavonoid aglycones. This paper is concerned with the isolation, purification and characterization of these materials.

2',4'-Dihydroxychalcone was obtained as the major flavonoid of the exudate of *A. neovernicosa* (collections DS-9164 and DS-9972). Crystallization of the relevant combined fractions did not yield a pure substance, and the mixture containing 2',4'-dihydroxychalcone was subjected to prep. TLC on Si gel. After this step the purified compound could be crystallized from toluene-petrol giving light-yellow crystals, mp 142–143°. The UV and ¹H NMR spectral data were determined (Tables 1 and 2). The spectra indicate a chalcone with no substituent on the B ring and two *meta*-substituted hydroxyl groups on the A ring and are in agreement with those reported in the literature for 2',4'-dihydroxychalcone [2–4].

2',4'-Dihydroxy-3'-methoxychalcone was extracted as a light-yellow substance, by prep. TLC on Si gel.

MS *m/z* (rel. int.): 270 [M]⁺ (76), 269 [M – 1]⁺ (12), 255 [M – Me]⁺ (8), 193 [M – C₆H₅]⁺ (24), 166 ['Pic A']⁺ (83), according to [5], 152 (35), 138 (100), 121 (21), 104 ['Pic B'] (17), 103 ['Pic I']⁺ (29), 77 (27). The mass spectral data indicate a chalcone with two hydroxyls and one methoxyl [6], the B-ring being unsubstituted. This is confirmed by the NMR spectral data (Table 2). From the NMR signals it is obvious that all three substituents are adjacent. The compound under investigation thus could be either 2',3' - dihydroxy - 4' - methoxychalcone or 2',4' - dihydroxy - 3' - methoxychalcone. The UV spectrum (Table 1) indicates the absence of an *o*-dihydroxy group and the data are in accordance with those reported for the second possibility (except for UV with AlCl₃). By direct comparison with authentic larrein, it was found that the compound 4' - hydroxy - 2' - methylchalcone was isolated in relatively small quantity. The UV and ¹H NMR spectra were determined (Tables 1 and 2). MS *m/z* (rel. int.): 254 [M]⁺ (45), 253 [M – 1]⁺ (18), 226 (13), 151 ['Pic A']⁺ (39), 136 (26), 121 (33). Signals in ¹³C NMR: 188.66 (carbonyl carbon), 162.92 and 160.59 (C-4'/C-6'), 140.51 (β-carbon), 134.97 (C-1), 132.25 (C-2'), 129.91 (C-4), 128.83 (C-2/C-6), 128.08 (C-3/C-5), 127.27 (α-carbon), 119.81 (C-1'), 107.95 (C-3'), 99.23 (C-5'), 55.59 (OMe). Together, the spectral data establish that the compound isolated was 4' - hydroxy - 2' - methoxychalcone.

7-Hydroxyflavanone was obtained as a greyish material, mp 176°. It was detected only on TLC plates with indicator for UV₂₅₄. The UV spectrum (Table 2) favours the flavanone structure. MS *m/z* (rel. int.): 240 [M]⁺ (34), 239 [M – 1]⁺ (17), 163 ['Pic F']⁺ (32), according to ref. [5], 151 (100), 137 ['Pic D']⁺ (20), 136 ['Pic A']⁺ (59), 108 ['A' – 28]⁺ (71), 104 ['Pic B']⁺ (43), 103 (29). Based on these data and the ¹H NMR (Table 1), the isolated compound was identified as 7-hydroxyflavanone.

Table 1. UV data for flavonoid compounds from *Acacia neovernicensa*

Compound	EtOH λ_{\max} nm	AlCl ₃ λ_{\max} nm	NaOEt λ_{\max} nm	NaOAc λ_{\max} nm	NaOAc- H ₃ BO ₃ λ_{\max} nm
2',4'-Dihydroxychalcone	345, 318, (260)	(410), 354, (315)	400, (297), 278	365, 305	358, 308
4'-Hydroxy-2'-methoxychalcone	(335), 306, (225)	308	392, 296, (270)	(335), 303	(335), 300
2',4'-Dihydroxy-3'-methoxychalcone	342, 225	358	406, (300) 265	404, 305, 227	340, 314 282
7-Hydroxyflavanone	310, 275 232	308, 230	337, 255	337, 257	322, 276

Three additional constituents were identified by direct comparison on TLC in different solvents and by comparison of their UV spectra. They are 2',4',4-trihydroxychalcone (isoliquiritigenin), 5,7-dihydroxyflavanone (pinocembrin), and 5,7-dihydroxyflavone (chrysin). In one collection (DS-11448) small quantities of galangin, 3-methylkaempferol, and 3,3'-dimethylquercetin were found.

2',4'-Dihydroxychalcone was first isolated from 'whole plant' extracts of *Flemingia chappar* (Leguminosae) [7]. It is assumed that its distribution within the plant is restricted to certain parts. Later it was reported to co-occur with 7-hydroxyflavanone in aerial parts of *Larrea nitida* [3]. This statement was later revised with respect to the plant source which was determined to be *Zuccagnia punctata* (Leguminosae) [8]. Recently these two flavonoids were also found in leaves and stems of *Flourensia oolepis* (Compositae) [4]. 2',4' - Hydroxy - 3' - methoxychalcone has been isolated only once before [3] and since the plant source was not *Larrea* but *Zuccagnia*, the trivial name 'larrein' is now misleading. 4' - Hydroxy - 2' - methoxychalcone appears to be a new natural compound. The minor constituents, pinocembrin, chrysin and liquiritigenin, are not uncommon flavonoids. Except for isoliquiritigenin, all flavonoids reported from the resin of *A. neovernicensa* lack B-ring substitution.

A. neovernicensa is an additional example of the correlation between the existence of secretory structures, production of lipophilic exudates, and occurrence of free flavonoid aglycones [9]. Comparative TLC on polyamide indicates that different collections of *A. neovernicensa* exhibit a variety of flavonoid patterns. The implication of this observation requires further studies. In the five collections analysed hitherto (9164, 9972, 11441, 11448, 11450), 2',4'-dihydroxychalcone seems to be the only compound that is present in all (Table 3). In collection 11448, the flavonols galangin, 3-methylkaempferol and 3,3'-methylquercetin were encountered.

EXPERIMENTAL

Acacia neovernicensa (DS-9164, D. S. Seigler and G. Holstein, 38 miles south Cd. Juarez, Chihuahua, México; DS-9972, D. S. Seigler, S. G. Saupe and H. Welt, 6 miles east of

Langtry, Val Verde Co., Texas; DS-11441, D. S. Seigler and D. A. Young, 3 miles south of Marathon, Brewster Co., Texas; DS-11448, D. S. Seigler and D. A. Young, 7 miles south-west of Study Butte, Brewster Co., Texas and DS-11450, D. S. Seigler and D. A. Young, 45 miles north of Study Butte, Brewster Co., Texas; voucher specimens deposited in the University of Illinois Herbarium) leaf and stem material was washed with CHCl₃ to dissolve the resinous exudate and the yellow soln concd to yield a brownish sticky mass. This material was dried onto polyamide SC-6 (Macherey-Nagel, Düren) and placed on top of a column filled with the same absorbent in toluene. The column was eluted with toluene and increasing quantities of MeCOEt and MeOH. TLC was performed on polyamide DC-11 (Macherey-Nagel) with solvents A (toluene-petrol, bp 100-140°-MeCOEt-MeOH, 12:6:2:1) and B (toluene-dioxane-MeOH, 8:1:1) and on Si with solvents C (toluene-MeCOEt, 9:1) and D (toluene-dioxane-HOAc, 90:25:4). Prep. TLC was carried out on Si plates with a concentrating zone (SILGUR-25, UV₂₅₄; Macherey-Nagel). The chromatograms were visualized by spraying with 'Naturstoffreagenz A' (β -aminodiethyl ether of diphenyl boric acid; C. Roth, Karlsruhe; 0.5% in MeOH).

Pinocembrin and chrysin were available as authentic samples, isolated from *Populus* bud excretions [10]. 2',4',4-Trihydroxychalcone was a gift from G. Schultz, Hannover. Samples of 2',4'-dihydroxychalcone and 2',4' - dihydroxy - 3' - methoxychalcone were kindly supplied by R. Pederiva, San Luis, Argentina.

Mass spectra were recorded on Varian MAT 311A at the Institut für Organische Chemie der TH Darmstadt. ¹H NMR spectra were run on a Bruker HFX 90 and the ¹³C NMR spectrum recorded on a Bruker WH 300, both at the Organisch-Chemisches Institut der Universität Heidelberg.

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Table 2. NMR spectral data for flavonoid aglycones from *Acacia neovernicosa**

Compound	OH	OH	H-2,4,6	H-3,5	H-3'	H-5'	H-6'	α	β	H-2,4,6,3,5,2', α,β	OMe
2',4'-Dihydroxychalcone	13.4 1H	10.86 1H	7.45m 3H	7.9m 2H	6.32d 1H	6.44dd $J = 8.5$ $J' = 2$	8.20d $J = 8.5$ 1H	7.84 $J = 16$ 1H	7.95 1H	—	—
4'-Hydroxy-2-methoxychalcone	—	10.3 1H	—	—	6.53-6.48 2H	—	—	—	—	7.7-7.3m 8H	3.88 3H
2',4'-Dihydroxy-3'-methoxychalcone	13.5 1H	— (exch.)	7.58m 3H	7.9m 2H	—	6.52d 1H	8.0d 1H	7.85 $J = 15$ 1H	7.8 $J = 15$ 1H	—	3.77 3H
7-Hydroxyflavanone	OH 10.6 1H	—	H-2',3',4',5',6' 7.5 5H	—	H-8 6.37d 1H	H-6 6.52d 1H	H-5 7.64d 1H	H-2 5.58dd $J_{23a} = 12$ Hz $J_{23b} = 3.5$ Hz	H-3 3.13dd $J_{2a3b} = 16.5$ Hz	—	—

*Spectra were recorded in DMSO- d_6 . Values are given in ppm (δ -scale) relative to TMS as an int. standard. Numbers in parentheses denote coupling constants in Hz; signals are singlets unless otherwise stated: *d* (doublet); *dd* (double doublet), *m* (multiplet).

Table 3. Distribution of flavonoids in the exudate of *Acacia neovernicosa* collections

Flavonoid	DS-9164	DS-9972	DS-11441	DS-11448	DS-11450
2',4'-Dihydroxychalcone	++	++	++	+	++
4'-Hydroxy-2'-methoxychalcone	—	+	(+)	—	—
2',4'-Dihydroxy-3'-methoxy-chalcone (larrein)	++	—	—	—	++
Isoliquiritigenin	(+)	(+)	—	—	—
7-Hydroxyflavanone	+	+	+	—	+
Pinocembrin	(+)	+	+	—	+
Chrysin	+	+	+	—	+
Galangin	—	—	—	+	—
3-Methylkaempferol	—	—	—	(+)	—
3,3'-Dimethylquercetin	—	—	—	+	—

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