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DIHYDROFLAVONOLS OF *ARTEMISIA DRACUNCULUS*

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Key Word Index—*Artemisia dracunculus*, Compositae, deuterated flavonoids, dihydroflavonols

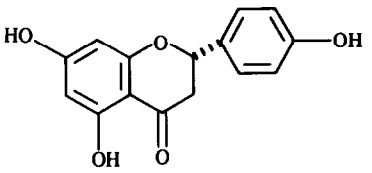
Abstract—The two dihydroflavonols, 3,5,4′-trihydroxy-7-methoxyflavanone and 3,5,4′-trihydroxy-7,3′-dimethoxyflavanone, and naringenin were isolated from aerial parts of *Artemisia dracunculus* The mass spectrum of 3,5,4′-trideuteroxy-7-methoxyflavanone is described as an example of the usefulness of deuteration in the analysis of certain flavonoids

INTRODUCTION

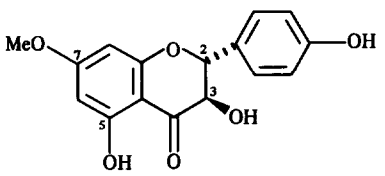
Several flavonoids have been identified from species of *Artemisia* L, one of the largest and most widely distributed of approximately 60 genera in the tribe Anthemideae of the Asteraceae (Compositae) [1] In a continuing study of the phytochemistry of the Asteraceae of British Columbia we report the isolation of a flavanone and two dihydroflavonols in *A dracunculus* L var *dracunculus*, a species which has hitherto not been examined The flavanone was characterized as naringenin, 5,7,4′-trihydroxyflavanone (**1**) The dihydroflavonols are 3,5,4′-trihydroxy-7-methoxyflavanone (**2**) and 3,5,4′-trihydroxy-7,3′-dimethoxyflavanone (**3**), these compounds occur in a non-glycosylated form Analysis by mass spectrometry of 3,5,4′-trideuteroxy-7-methoxyflavanone (**2a**) showed deuterium labelling to be a useful technique for the identification and location of hydroxyl groups in certain flavonoids

RESULTS AND DISCUSSION

Free dihydroflavonols and flavanones, common in woody plants, may also occur in herbaceous species as minor constituents [2] Naringenin (**1**) and 3,5,4′-



1



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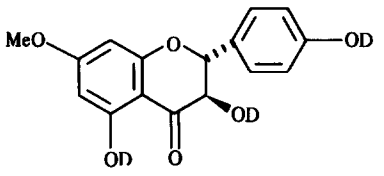
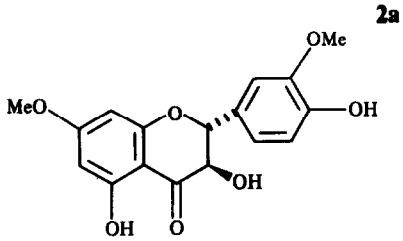


2a



3

trihydroxy-7-methoxyflavanone (**2**) were isolated as a 5 1 mixture Complete separation of **1** and **2** was achieved with preparative TLC on silica gel employing chloroform–methanol (7 1) ^{1}H NMR as well as other chemical and spectroscopic analyses supported the proposed structures of **1** and **2** [3, 4]

Mass spectral data for 7-methoxy substituted dihydroflavonols such as **2** and **3**, present characteristic and unique fragments useful in the identification of this substituent in these molecules Crystallization of **2** from $CDCl_3$ and a drop of D_2O afforded pure 3,5,4′-trideuteroxy-7-methoxyflavanone (**2a**), its mass spectrum enabled us to identify and confirm the location of the three hydroxyl groups in the main fragments as well as in some of the less intense ones by comparison with the high resolution measurements of the undeuterated analog (**2**) (Fig 1) An increase in three mass units in the molecular ion as well as in the fragment at *m/z* 273 of **2** revealed incorporation of three deuterium atoms and clearly demonstrated the isotopic composition of **2a** (Scheme 1) Ions at *m/z* 167 (base peak) and 179 give information on ring A, the former being a protonated ion of a typical fragment from a retro-Diels–Alder cleavage [5] They increased by two and one mass units, respectively in the fragments derived from **2a** Since the fragment at *m/z* 167 (from **2**) has one hydroxyl group at C-5 and after deuteration has increased by two mass units, the source of the other proton in the ketene protonated residue must have come from the hydroxyl

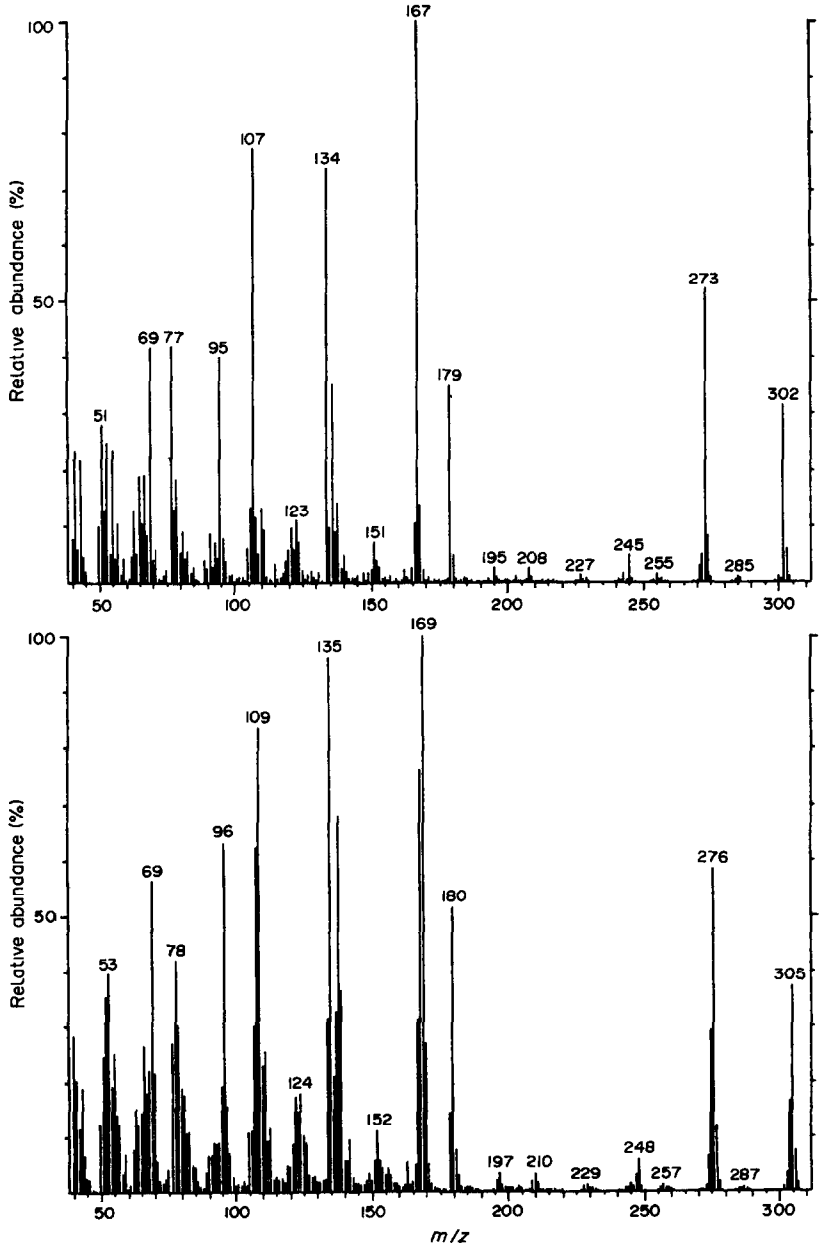
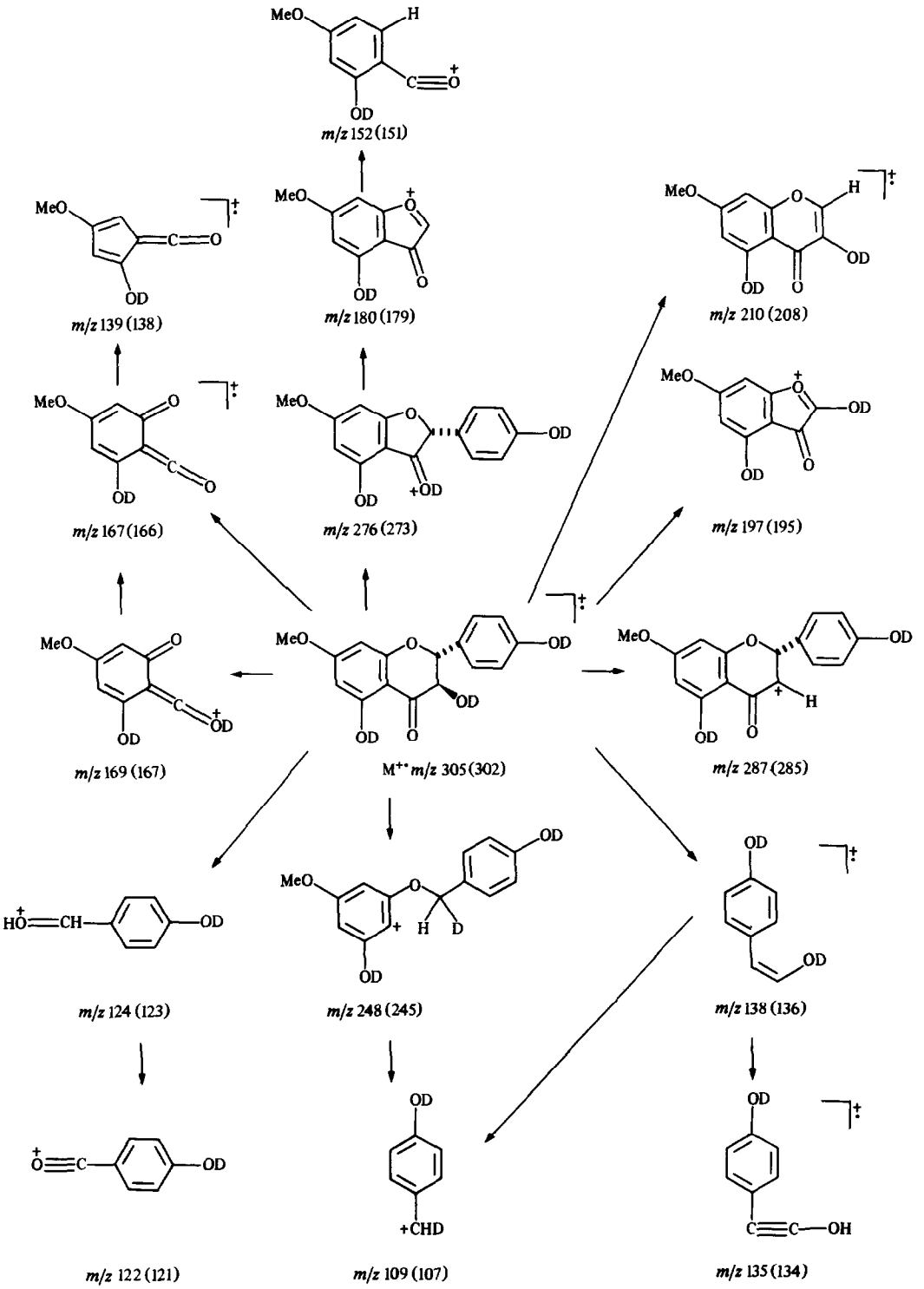


Fig 1 Mass spectra of 3,5,4′-trihydroxy-7-methoxyflavanone (**2**) and 3,5,4′-trideuteroxy-7-methoxyflavanone (**2a**)



Scheme 1 Mass spectral fragmentation of 3,5,4'-trideuteroxy-7-methoxyflavanone (**2a**) The number in brackets refers to the corresponding fragments of the undeuterated compound

group at C-3 thus increasing the mass of this ion to *m/z* 169 in **2a** The ion at *m/z* 179 is shifted one mass unit higher, as would be expected, having only one hydroxyl group in its structure

Losses of carbon monoxide and a hydrogen radical from the molecular ion produces the ion at *m/z* 273 for **2** This was shifted three mass units higher to *m/z* 276 in **2a** In this process the hydrogen radical involved probably

comes from the fragmentation of the C-3–H-3 bond rather than from the homolytic rupture of the hydroxyl group at C-3

Formation of the ion at *m/z* 136, arising from the retro-Diels–Alder process, gives information about ring B The mass increase by two units (to *m/z* 138) in **2a** is due to the presence of two deutero-exchangeable hydroxyl groups Since this fragment (*m/z* 136) must be at least in part, if not all, a precursor of the ion at *m/z* 134 after dehydrogenation, the fragment at *m/z* 134 should also be two mass units higher However, we see an increase of only one mass unit to *m/z* 135 in the deutero-analog (**2a**) Keto-enol [6] tautomerization of the ion at *m/z* 136 may, in part, account for this difference as well as for the generation of the ion at *m/z* 107 ('tropylium structure') which, unexpectedly, increased to *m/z* 109 There is only one deutero-exchangeable hydroxyl group in its structure and accordingly this ion should be one mass unit higher than the undeuterated fragment (Scheme 2)

The ion $[C_6H_7O]^+$, *m/z* 95, is also prominent in the mass spectrum of **2** and its origin is related to ring B It is phenolic in nature, an increase of one mass unit in **2a** (*m/z* 96) is expected It is often observed that the daughter ions retain the isotopic label in abundances which must have resulted from a complete loss of positional identity of the labelled and unlabelled atoms within the precursor ion This randomization process of hydrogen and deuterium atoms also occurs as shown by the appearance of a fragment at *m/z* 77 $[C_6H_5]^+$ in **2** and the corresponding ion at *m/z* 78 $[C_6H_4D]^+$ in **2a** (Fig 1)

Minor fragments of the overall pattern are typical and may also be considered diagnostic especially in mixtures of flavonoids Some of them may also contribute to the enhancement of the intensities of the major fragments as shown in Scheme 1 by further fragmentation of their corresponding ions

The similarity in the proton signals on rings A and C of the dihydroflavonols **2** and **3** is shown in the ^{1}H NMR spectra of these two compounds (Table 1) A complete assignment for the proton signals on ring B of **3** was made after degradation of this compound by alkali fusion [7] TLC on silica gel 60 F_{254} of the fusion products yielded vanillic acid thus confirming the structure of **3** as 3,5,4'-trihydroxy-7,3'-dimethoxyflavanone ^{13}C NMR assignments for **3** in acetone-d_6 (Table 2) were also consistent with this structure They were based on data obtained from single frequency off-resonance decoupled spectrum (SFORD) as well as by comparison with earlier data on dihydroflavonols [8] Although **3** has been previously isolated from *Artemisia pygmaea* [1], the reported value for UV λ_{max}^{MeOH} = 272 nm is considerably lower than that obtained by us (UV λ_{max}^{MeOH} = 288 nm)

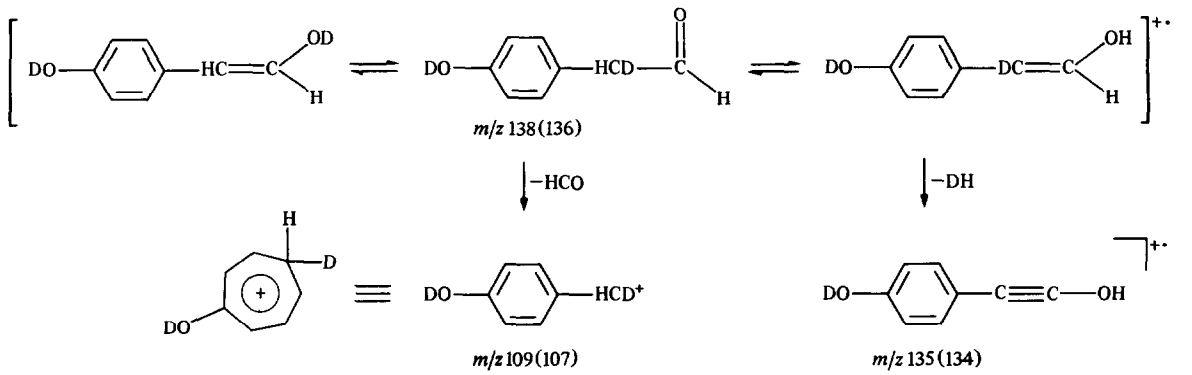
EXPERIMENTAL

^{1}H NMR spectra were determined with a Bruker WP-80 and a WH-400 Fourier Transform NMR spectrometer Chemical shifts are reported in δ units (ppm) relative to TMS ^{13}C NMR spectra were obtained using a Bruker WH-400 Fourier Transform NMR spectrometer operating at 100 6 MHz with Me_2CO-d_6 as solvent MS were recorded on a Finnigan 1020 GC/MS, and a KRATOS-AEI MS 50 High Resolution mass spectrometer operating at 70 eV Mps are uncorr

Isolation of **1**, **2** *and* **3** Freeze dried aerial parts of *Artemisia dracunculus* (170 g) collected at Lytton, British Columbia in May, 1983, were ground and extracted with $CHCl_3$ The crude extract was evaporated to dryness and taken up in 95% EtOH, precipitated with 4% aq $Pb(OAc)_2$ soln and filtered The filtrate was concd *in vacuo*, extracted with $CHCl_3$, the extract treated with activated charcoal and finally dried over dry $MgSO_4$ Evaporation of solvent yielded 3 7 g of a brown tar which was dissolved in $CHCl_3$ and chromatographed on silica gel (Merck 70–230 mesh) Eight fractions were collected with solvent polarity changing from $CHCl_3$, $CHCl_3$–Me_2CO (6 1) and $CHCl_3$–Me_2CO (7 3) All three compounds gave a pink colour with Mg–HCl in MeOH [9]

Identification of 3,5,4'-*trihydroxy*-7,3'-*dimethoxy flavanone* (**3**) Fraction 5 gave 53 mg of **3** recrystallized from Me_2CO–petrol, R_f (**3**) 0 43 in $CHCl_3$–Me_2CO (6 1), located by shortwave UV light and by the deep red colour formed after spraying with vanillin followed by heating [10], mp 184–186° (lit [1] mp 182–184°), IR ν_{max}^{nujol} cm^{-1} 3440 (OH), 1645 (C=O), 1583 (aromatic), UV λ_{max}^{MeOH} nm 333 (sh), 288, MS *m/z* (rel int) 332.0899 $[M]^+$ (50), 303 $[C_{16}H_{15}O_6]$ (67), 275 $[C_{15}H_{15}O_5]$ (8), 179 $[C_9H_7O_4]$ (61), 167 $[C_8H_7O_4]$ (100), 166 $[C_9H_{10}O_3, C_8H_6O_4]$ (80), 164 $[C_9H_8O_3]$ (92), 151 $[C_8H_7O_3]$ (33), 137 $[C_8H_9O_2]$ (84), 123 $[C_7H_7O_2]$ (25), 110 $[C_6H_6O_2]$ (19), 95 $[C_6H_7O]$ (76)

Alkali fusion of **3** Two mg of **3**, a pellet of KOH and a drop of H_2O were heated to 250° in a tube for 5 min On cooling, 1 ml of distilled H_2O was added and after acidification with HCl to pH 1, the products were extracted with 2 ml of Et_2O TLC along with standards of vanillic and isovanillic acids were run on silica gel 60 F_{254} using 6% HOAc in $CHCl_3$ R_f [vanillic acid] = 0 68 and R_f [isovanillic acid] = 0 59 Spots on TLC were visualized under



Scheme 2

Table 1 ^{1}H NMR spectral data of **1**, **2** and **3***

	1†	2†	3‡
H-2	5 45 *dd*	5 05 *d*	5 03 *d*
H-3	2 98 *dds*	4 55 *dd*	4 55 *dd*
H-6	5 98 *s*	6 05 *d*	6 07 *d*
H-8	5 98 *s*	6 15 *d*	6 13 *d*
H-2′	7 40 *d*	7 45 *d*	7 08 *d*
H-3′	6 93 *d*	6 90 *d*	—
H-5′	6 93 *d*	6 90 *d*	6 99 *d*
H-6′	7 40 *d*	7 45 *d*	7 05 *dd*
OH-3	—	3 47 *d*	3 45 *d*
OH-5	12 19 *s*	11 18 *s*	11 18 *s*
OH-7	9 55 *s*	—	—
OH-4′	8 50 *s*	4 90 *s*	5 71 *s*
OMe-7	—	3 83 *s*	3 81 *s*
OMe-3′	—	—	3 93 *s*

*TMS as internal standard, δ

†Measured at 80 MHz in acetone-d_6 (**1**) and $CDCl_3$ (**2**)

‡Measured at 400 MHz in $CDCl_3$

s, Singlet, *d*, doublet, *dd*, doublet of doublet, *dds*, doublet of doublets

J (Hz) **1** 2, 3 = 12 5, 3 5, 3, 2 = 17, 12 5, 3 5, 2′, 3′ = 8 5, 5′, 6′ = 8 5 **2** 2, 3 = 12, 3, OH-3 = 1 5, 6, 8 = 2 5, 2′, 3′ = 8 5, 5′, 6′ = 8 5 **3** 2, 3 = 12, 3, OH-3 = 1 5, 6, 8 = 2 5, 2′, 6′ = 2 0; 5′, 6′ = 8 5

Table 2 ^{13}C NMR spectral data of **3***

Carbon number	3
2	84 9 *d*
3	73 3 *d*
4	198 6 *s*
5	164 9 *s*
6	96 0 *d*
7	169 5 *s*
8	94 9 *d*
9	164 1 *s*
10	102 2 *s*
1′	129 6 *s*
2′	112 7 *d*
3′†	148 3 *s*
4′†	148 6 *s*
5′	115 7 *d*
6′	122 3 *d*
OMe	56 4, 56 6 *q*, *q*

*Run at 100 6 MHz in acetone-d_6, δ

†Assignments may be reversed

s, Singlet, *d*, doublet, *q*, quartet

shortwave UV and the compounds were characterized by UV, NMR and MS after elution in Et_2O

Identification of naringenin (**1**) *and 3,5,4′-trihydroxy-7-methoxy-flavanone* (**2**) Fractions 7 and 8 afforded 50 mg of a mixture of **1** and **2** They were separated on silica gel 60 F_{254} prep TLC plate using $CHCl_3$–MeOH (7 1), R_f (**1**) 0 65 and R_f (**2**) 0 75 Compounds were identified with shortwave UV and with the vanillin reagent Compound **1** gave a deep red colour and **2** a greenish colour Compound **1**, 7 5 mg, recrystallized from MeOH–H_2O, gave mp 250–251° (lit [3] mp 250–251°), IR ν_{max}^{nujol} cm^{-1} 3400 (OH), 1630 (C=O), 1602, 1519 (aromatic), UV λ_{max}^{MeOH} nm 324 (sh), 290; MS *m/z* (rel int) 272 0679 $[M]^+$ (73), 271 $[C_{15}H_{11}O_5]$ (45), 179 $[C_9H_7O_4]$ (59), 166 $[C_8H_6O_4]$ (66), 153 $[C_7H_5O_4]$ (100), 152 $[C_7H_4O_4]$ (57), 124 $[C_6H_4O_3]$ (66), 120 $[C_8H_8O]$ (95), 119 $[C_8H_7O]$ (54), 107 $[C_7H_7O]$ (59) Compound **2**, 42 5 mg, recrystallized from $CHCl_3$, gave mp 181–182° (lit [4] mp 181–183°), IR ν_{max}^{nujol} cm^{-1} 3400 (OH), 1638 (C=O), 1574 (aromatic) UV λ_{max}^{MeOH} nm 335 (sh), 290 MS *m/z* (rel int) 302 0789 $[M]^+$ (32), 273 $[C_{15}H_{13}O_5]$ (51), 179 $[C_9H_7O_4]$ (33), 167 $[C_8H_7O_4]$ (100), 136 $[C_8H_8O_2]$ (33), 134 $[C_8H_6O_2]$ (72), 107 $[C_7H_7O]$ (78), 95 $[C_6H_7O]$ (40), 77 $[C_6H_5]$ (43)

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