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PROTOCATECHUIC ACID DERIVATIVES FROM *HEMIZONIA LOBBII*

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Key Word Index—*Hemizonia lobbii*; Compositae; protocatechuic acid derivatives.

Abstract—From the external leaf resin of *Hemizonia lobbii*, 3,4-dihydroxy-5-(2-isopentenyl)-benzoic acid (5-prenylprotocatechuic acid) and two related compounds have been isolated.

INTRODUCTION

From *Hemizonia* species, a genus which is endemic to California and Baja California Norte, so far only labdane type diterpenes, chromene derivatives and flavonoids have been isolated [1–3]. We now wish to report on the isolation and structural elucidation of three protocatechuic acid derivatives isolated from *Hemizonia lobbii*.

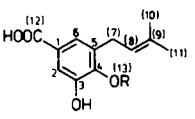
RESULTS AND DISCUSSION

The air dried leaves and stems of *H. lobbii* were dipped in methanol to wash off external resin. After CC on Sephadex LH-20 three fractions were collected which after spraying with Naturstoffreagenz A exhibited a characteristic blue colour under $UV_{366\,nm}$.

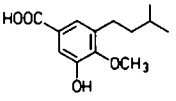
3-*Hydroxy*-5-(2-*isopentenyl*)-4-*methoxybenzoic acid* (1)

Exact mass measurement gave an elemental formula $C_{13}H_{16}O_4$. The ^1H NMR spectrum (Table 1) shows the presence of two hydrogens bound to a benzene ring in a *meta* position, an aromatic methoxyl group and a pattern typical of a $-CH_2-CH{=}CMe_2$ residue. Thus, the atoms $C_{12}H_{14}O$ have been accounted for; the remaining ones add up to a hydroxyl and a carboxyl group. Regarding the arrangement of the four substituents some deductions can be made from the mass spectrum. The carboxyl group can be neighbouring neither the hydroxyl nor the isopentenyl group since in either case loss of water from $[M]^+$ would have been expected from the *ortho*-effect [4], which is not observed. (Since the co-occuring **2** which instead of the methoxyl carries a second hydroxyl group does not show an $[M-H_2O]^{+\cdot}$ ion either, the neighbourhood of carboxyl and methoxyl can also be excluded.) The isopentenyl group must have at least one unsubstituted *ortho*-position since the main fragment of **3** (dihydro-**1**) is due to $[M-C_4H_8]^+$ arising from a McLafferty rearrangement which is not observed if both *ortho*-positions of an alkyl benzene derivative are substituted [5, 6]. This is confirmed by the ^1H NMR spectrum; irradiation at δ3.2 (benzylic CH_2 group) results in a sharpening of the signal at δ7.18 due to the disappearance of the ^4J coupling between the CH_2 and the neighbouring aromatic CH protons. From this either structure **1** can be deduced or an isomer where hydroxyl and methoxyl have been exchanged.

A confirmation of the structural conclusions arrived at, and a final decision regarding the position of the methoxyl



1 R = CH_3
2 R = H



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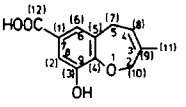

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Table 1. ^1H NMR data (DMSO-d_6, TMS, δ-values) [multiplicity, chemical shift, *J* (Hz)] for compounds **1**, **2** and **4**

H			**1**			**2**			**4**
2	*d*	7.26	2.1 (H-6)	*d*	7.25	2.0 (H-6)	*d*	7.24	2.0 (H-6)
6	*d*	7.18	2.1 (H-2)	*d*	7.18	2.0 (H-2)	*d*	7.18	2.0 (H-2)
7	*d*	3.22	7.3 (H-8)	*d*	3.22	7.3 (H-8)	*d*	3.25	7.4 (H-8)
8	*tm*	5.24	7.3 (H-7)	*tm*	5.28	7.3 (H-7)	*tm*	5.47	7.4 (H-7)
			~1 (H-10, H-11)			~1 (H-10, H-11)			~1 (H-10, H-11)
10	*br d*	1.68*	~1 (H-8)	*br d*	1.70*	~1 (H-8)	*br d*	3.79	~1 (H-8)
11	*br d*	1.65*	~1 (H-8)	*br d*	1.66*	~1 (H-8)	*br d*	1.63	~1 (H-8)
13	*s*	3.74							

*Assignment may be reversed.
Integration agrees with the number of H-atoms present in the respective groups.

Table 2. ^{13}C NMR data (DMSO-d_6, TMS, δ-values) [multiplicity, chemical shifts, calculated [7] shifts for the aromatic C in parentheses, J(Hz)] for compounds 1 and 4

C		1			4
1	*t*	127.9 (125.3)*			127.5
2	*dd*	113.7 (115.9)	160 (H-2) 7 (H-6)	*dd*	114.1†
3	*s*	144.5 (141.1)		*s*	144.5
4	*m* (*t*)‡	148.0 (152.3)	7 (H-2, H-6)		147.6
5	*s*	119.6 (129.7)		*s*	121.0
6	*dm*	122.4 (124.2)	153 (H-6)	*d*	121.4†
7	*t*	27.9	127 (H-7)	*t*	27.5†
8	*dm*	121.8	160 (H-8)	*d*	122.1†
9	*m*	131.6			135.9
10	*q*	17.6‖	125 (H-10)	*t*	66.2†
11	*q*	25.5‖	121 (H-11)	*q*	13.6†
12	*x*§	166.3			167.6
13	*q*	51.5	147 (H-13)		

*J < 2 Hz could not be determined accurately and not quoted.

†Due to the unfavourable signal-to-noise ratio coupling constants could not be determined accurately and not quoted.

‡Upon irradiation at 3.7 ppm (H-13).

§X-part of an ABX-pattern (see text).

‖In the literature the reverse assignment of the two methyl groups of an isopentenyl residue may also be found (cf., e.g. refs [9] and [10]), although the correlation 17.6 ppm for the *cis* and 25.6 ppm for the *trans* Me has been settled unambiguously by deuterium labelling [12].

group could be obtained from the ^{13}C NMR spectrum (Table 2). The chemical shifts agree with the literature data [7] including the values for the aromatic carbons obtained by increment calculation (for the isopentenyl residue see, e.g. [8, 9]). The substitution pattern can be deduced from C,H-coupling data [10]: C-1 yields a triplet with a very small (*ortho*-H)^2J coupling constant. This is confirmed by C-12 which forms the X-part of an ABX pattern, A and B being H-2 and H-6. C-2 yields a double doublet (160 Hz for the C,H (*ipso*) and 7 Hz for the C,H (*meta*) coupling with H-6), C-6 in turn a double multiplet (153 Hz for the C,H (*ipso*) coupling; long range coupling with H-7). The multiplet of C-6, upon irradiation of the signal of the methoxyl group (δ3.7) collapses to a triplet with ^3J = 7 Hz. This indicates the presence of H atoms at both *meta* positions (C-2 and C-6) with respect to the C carrying the methoxyl group (C-4). For the alternative structure (OH at C-4 and OMe at C-3) C-3 would show coupling with the H atoms at C-2 ($^2J \sim 1$ Hz) and at C-6 ($^4J \sim 1$ Hz); upon irradiation at the signal of the methoxyl group it should, therefore, give rise to a slightly broadened singlet. The isomeric arrangement of substituents can thus be excluded.

The fragmentation patterns of 1 and 3 require a brief mention. The most important process for 1 is the loss of $^{\cdot}C_4H_7$ from the isopentenyl group (m/z 181). The unexpected elimination of $^{\cdot}$OMe (m/z 205) has also been observed with *p*-allyl anisol [11] and seems, therefore, to be typical for these systems. The mass spectrum of 3 shows, as mentioned above, loss of C_4H_8 by McLafferty rearrangement (m/z 182) accompanied by that of $^{\cdot}C_4H_9$ (benzylic cleavage).

3,4-Dihydroxy-5-(2-isopentenyl)-benzoic acid (2)

Exact mass measurement gives an elemental composition of $C_{12}H_{14}O_4$. The main fragment, as observed for 1, is formed by loss of C_4H_7 (m/z 167). The ^{1}H NMR spectrum corresponds to that of 1 except for the missing methoxyl signal (see Table 1).

2,5-Dihydro-9-hydroxy-3-methyl-1-benzoxepin-7-carboxylic acid (4)

Exact mass measurement gives an elemental composition of $C_{12}H_{12}O_4$. The only features of importance in the mass spectrum are the loss of $^{\cdot}$Me (m/z 205) and of CO_2 (m/z 176) from the $[M]^+$ which indicates the absence of a free isopentenyl side chain (cf. 1 and 2). This is confirmed by the ^{1}H NMR spectrum which corresponds to that of 1 and 2 with the exception that one of the methyl signals is replaced by that for a CH_2-group with a chemical shift of δ3.79. The relatively high field value (the CH_2-signal for, e.g. allyl phenyl ether occurs at δ4.5 ($CHCl_3$) [13], that for 2,5-dihydrooxepin in the region δ4.15–4.50 (CCl_4) [14]) can partially be explained by a solvent effect (DMSO; in CH_3OD solution it is shifted to δ3.96) and may otherwise be due to electronic and steric effects caused by the special cyclic structure. Also the ^{13}C NMR spectrum of 4 reflects the close similarity with 1 differing only in the replacement of a methyl-quartet by a CH_2-triplet.

Vouchers of *Hemizonia lobbii* (identified by Dr. B. D. Tanowitz) are deposited in the herbarium of the University of California, Santa Barbara.

EXPERIMENTAL

MS were determined at 70 eV, direct inlet, ^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz.

Isolation of 1, 2 *and* 4. Air dried leaves and stems of *H. lobbii* Greene were dipped in MeOH for *ca* 1 min to remove external resin constituents. The crude extract was separated by CC on Sephadex LH-20 with MeOH as eluent. The fractions were checked by TLC on Polyamide DC6 (C_6H_6–MeCOEt–MeOH–H_2O, 60:22:20:3). Compounds 1, 2 and 4 showed a characteristic blue colour under $UV_{366\,nm}$ after spraying with Naturstoffreagenz A (1% diphenylboric acid 2-amino-ethyl ester in MeOH). Final purification was achieved by prep. TLC on polyamide and subsequently on Sephadex LH-20 with MeOH as eluent. From 100 g dried plant material 20 mg 1, 3 mg 2 and 13 mg 4 were obtained as glassy solids.

3-Hydroxy-5-(2-isopentenyl)-4-methoxybenzoic acid (1). ^{1}H and ^{13}C NMR: see Tables 1 and 2. MS m/z (% rel. int.): 236.1055 $[M]^+$ (86) (calc. 236.1049 for $C_{13}H_{16}O_4$), 211 $[M-Me]^+$ (6), 209 $[M-OH]^+$ (4), 207 $[M-CHO]^+$ (6), 205 $[M-OMe]^+$ (19), 189 $[M-Me-MeOH]^+$ (16), 181.0497 $[M-C_4H_7]^+$ (100) (calc. 181.0501 for $C_9H_9O_4$), 180 $[M-C_4H_8]^+$ (88), 177 $[205-CO]^+$ (13), 163 (6), 162 (6), 161 (7), 149 (19). UV λ_{max}^{MeOH} nm: 265 and 300.

3-Hydroxy-5-isopentenyl-4-methoxybenzoic acid (3). From 1 by catalytic hydrogenation with PtO_2 in EtOH. MS m/z (% rel. int.): 238 $[M]^+$ (34), 207 $[M-OMe]^+$ (14), 182 $[M-C_4H_8]^+$ (100), 181 $[M-C_4H_9]^+$ (44), 167 (4), 164 (3), 163 (6), 151 [182 $-OMe]^+$ (7), 150 (15), 149 (7), 123 $[151-CO]^+$ (46), 122 (13).

3,4-Dihydroxy-5-(2-isopropenyl)-benzoic acid (2). ^{1}H NMR: see Table 1. MS m/z (% rel. int.): 222.0899 $[M]^+$ (79) (calc. 222.0892 for $C_{12}H_{14}O_4$) 167.0331 $[M-C_4H_7]^+$ (100) (calc. 167.0344 for $C_8H_7O_4$), 166 $[M-C_4H_8]^+$ (68). UV λ_{max}^{MeOH} nm: 265 and 300.

2,5-Dihydro-9-hydroxy-3-methyl-1-benzoxepin-7-carboxylic acid (**4**). ^1H and ^{13}C NMR: see Tables 1 and 2. MS *m/z* (% rel. int.): 220.0739 $[M]^+$ (40) (calc. 220.0736 for $C_{12}H_{12}O_4$), 205.0493 $[M-Me]^+$ (100) (calc. 205.0501 for $C_{11}H_9O_4$), 176 $[M-CO_2]^+$ (24), 174 (9), 161 $[176-Me]^+$ (30), 157 (19), 143 (8), 129 (14), 115 (13). UV λ_{max}^{MeOH} nm: 265 and 300.

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