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NEW PRENYLATED PHENOLICS FROM *PIPER AURITUM*

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Key Word Index—*Piper auritum*; Piperaceae; leaves; chromenoic acid; chromanoic acid; benzoic acid derivatives; leafcutter ants; ant-repellents.

Abstract—Four prenylated phenolics have been isolated from the leaves of *Piper auritum*, and characterized on the basis of their physical and spectral data.

INTRODUCTION

During our studies [1–3] of defense mechanisms in tropical plants avoided by the leafcutter ant *Atta cephalotes*, we screened the leaves of the shrub *Piper auritum* for novel secondary metabolites. Fractionation of the extracts of *P. auritum* leaves resulted in the isolation of four benzoic acid derivatives, which were characterized by their physical and spectroscopic data. Three of these compounds are new natural products, while the fourth [4-hydroxy-5-(*E*,*E*-farnesyl)benzoic acid] has only very recently been reported from another plant species. This report deals with the isolation and identification of these compounds.

RESULTS AND DISCUSSION

After extraction of the air-dried leaves with chloroform and concentration of the extract *in vacuo*, the residue was partitioned between hexane and methanol–water (1:1). The material from the hexane layer was fractionated by column and radial layer chromatography; four compounds were eventually isolated.

The high resolution mass spectrum of the first isolated compound indicated a molecular formula of $C_{22}H_{28}O_3$. Its IR spectrum shows a carbonyl absorption (1760 cm^{-1}), as well as the broad hydroxyl absorption (3400 cm^{-1}) typical of a carboxylic acid. When esterification gave a methyl ester derivative, the presence of a carboxylic acid functional group in the parent compound was confirmed. The UV spectrum, with absorption maxima at 238, 283, 305, and 318 nm, then suggested a chromenoic acid moiety [4, 5].

The ^{1}H NMR spectrum of compound **1** contains a set of three coupled aromatic resonances (δ6.79, 7.72, and 7.87, J_{ab} = 8.5 Hz, J_{bc} = 2 Hz), which suggested the presence of a 1,3,4-substituted benzene ring. A second set of downfield signals (δ6.39 and 5.61, J = 10 Hz) indicated an isolated *cis*-disubstituted olefin, as expected for a chromenoic acid nucleus. When one of the remaining resonances (δ1.55) was assigned to a methyl substituent, the rest of the ^{1}H NMR spectrum indicated that a 4,8-dimethyl-3,4-nonadienyl group was the second substitutent. The electron impact mass fragmentation pattern, which shows a base peak at m/z 189 ($[C_{11}H_9O_3]^+$, **2**) and a fragment ion at m/z 151 $[C_{11}H_{19}]^+$, reflects cleavage between the chromenoic acid nucleus and this large side chain.

The ^{13}C NMR data (Table 1) of **1** were in complete agreement with the above conclusions, including a carbinol carbon (80.10 ppm), an acid carbonyl resonance (177.77 ppm), and a *para* relationship between the chromenoic oxygen and the carboxyl group (i.e. at C-6). Furthermore, these assignments are in good agreement with those reported for dictyochromenol **3** [6], except, as expected, for the effects of the additional carboxylic acid

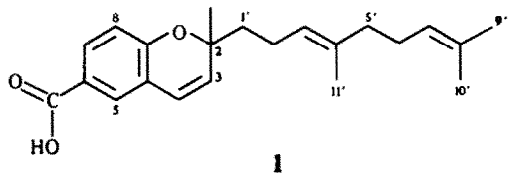
Table 1. ^{13}C NMR data for prenylated phenolics

Carbon no.	**1**	**3**[6]	**4**	**5**	**6**
CO_2H	171.8 *s*		170.7	172.2 *s*	172.1 *s*
1				127.8 *s*	126.9 *s*
2	80.1 *s*	78.2 *s*	76.0	115.0 *d*	130.5 *d*
3	130.0 *d*	131.0 *d*	153.9	147.9 *s*	115.7 *d*
4	122.2 *d*	124.5 *d*	29.7	142.9 *s*	159.5 *s*
5	128.8 *d*	113.3 *d*	124.1	120.6 *s*	121.6 *s*
6	121.4 *s*	149.3 *s*	128.2	125.0 *d*	132.5 *d*
7	131.9 *d*	115.5 *d*	111.4		
8	116.1 *d*	116.5 *d*	146.9		
8a	158.4 *s*	158.5 *s*	154.6		
4a	120.5 *s*	147.2 *s*	122.3		
1′	41.8 *t*	41.1 *t*	35.6	28.5 *t*	29.6 *t*
2′	22.5 *t*	22.7 *t*	23.1	121.8 *d*	120.8 *d*
3′	123.6 *d*	122.7 *d*	124.1	138.4 *s*	139.4 *s*
4′	135.5 *s*	135.3 *s*	135.2	39.7 *t*	39.7 *t*
5′	39.7 *t*	39.8 *t*	32.8	26.4 *t*	26.4 *t*
6′	26.7 *t*	25.7 *t*	25.9	123.8 *d*	123.3 *d*
7′	124.3 *d*	124.1 *d*	124.5	135.7 *s*	135.6 *s*
8′	131.3 *s*	131.3 *s*	131.3	32.1 *t*	39.7 *t*
9′	25.7 *q*	26.1 *q*	25.9	26.7 *t*	26.7 *t*
10′	17.7 *q*	17.7 *q*	17.7	124.4 *d*	124.3 *d*
11′	16.0 *q*	16.0 *q*	25.9	131.3 *s*	131.2 *s*
12′				25.6 *q*	25.7 *q*
13′				17.6 *q*	17.7 *q*
14′				23.5 *q*	16.3 *q*
15′				16.0 *q*	16.0 *q*
1″	27.2 *q*	26.8 *q*	111.4		

function. These facts support our assigned structure for compound **1**, which we have named piperochromenoic acid.

The ^{1}H NMR spectrum of the second isolated compound exhibited sufficient similarity to that of piperochromenoic acid to allow rapid identification of a nonadienyl group, albeit with a *Z*-olefin. However, the presence of a benzylic methylene resonance (δ 3.3), a terminal methylene resonance (*br s* at δ 4.81 and 4.91), and a downfield signal (δ 4.07, *dd*, J = 6.9, 6.21 Hz) coupled to the methylene protons of a 4,8-dimethyl-3,4-nonadienyl unit, indicated a chromanoic acid nucleus. Furthermore, differences in the aromatic resonances suggested A-ring modifications relative to piperochromenoic acid. A broad singlet (2 H at 7.45) was assigned to the hydrogens *ortho* to the carboxyl group. The absence of other signals in the aromatic region of the ^{1}H NMR spectrum, and the molecular formula obtained from the mass spectrum ($C_{22}H_{28}O_4$), required the presence of a hydroxyl group at C-8. Both the ^{13}C NMR spectrum, taking into consideration calculated values for the aromatic resonances [7] and model *Z*-olefins, and the mass fragmentation pattern were in complete agreement with structure **4** for this compound, which we have named piperochromanoic acid.

The third isolated compound also exhibited the spectral features of a substituted benzoic acid. Its mass spectrum gave a $[M]^+$ at m/z 358 ($C_{22}H_{30}O_4$), and the presence of a carboxylic acid function was indicated by IR absorptions at 1760 and 3400 cm^{-1}. The ^{1}H NMR spectrum contained only two aromatic protons, with chemical shifts and



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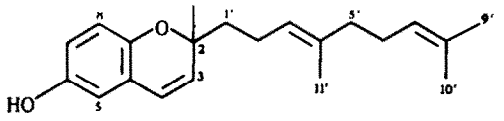

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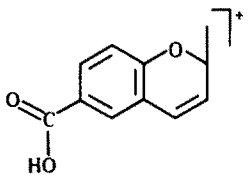

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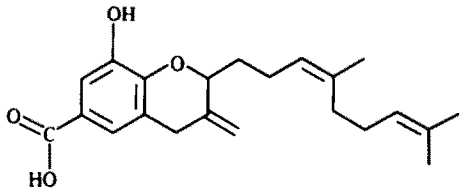

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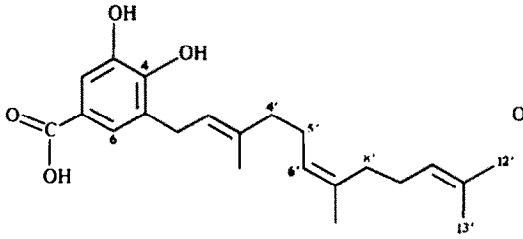

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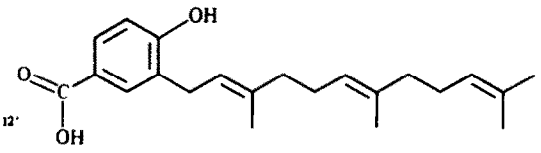

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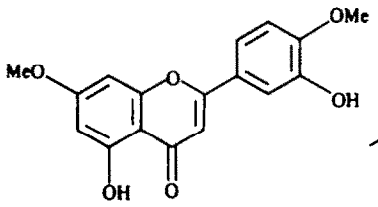

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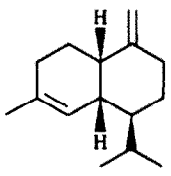

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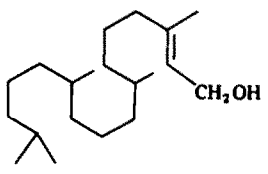

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splitting pattern typical of H-2 and H-6 of a 3,4,5-substituted benzoyl unit. Furthermore, the ^{1}H NMR spectrum revealed the presence of a farnesyl chain. On the basis of these data and the ^{13}C NMR spectrum (Table 1), this compound was assigned structure **5**, 3,4-dihydroxy-5-(*E,Z*-farnesyl)benzoic acid.

Finally, the fourth isolated compound gave a mass spectrum indicative of a monohydroxy analogue of compound **5**. When this tentative conclusion was supported by its IR and ^{1}H NMR data we assigned structure **6** to this compound. This was confirmed by comparison with literature data [8] recently reported for 4-hydroxy-5-(*E,E*-farnesyl)benzoic acid. The ^{13}C NMR spectrum, which had not been reported before, agrees with the assignment of *E*-olefin stereochemistry.

In addition to these new benzoic acid derivatives, several other compounds were isolated from *P. auritum* leaves. From the most polar fractions of the $CHCl_3$ extract, a phenolic compound exhibiting flavone UV characteristics was isolated. It was identified as 7-methoxy-3'-hydroxy-4'-methoxyflavone (**7**) from its mass spectrum and NMR features [9], and from the observation that acetylation caused a pronounced shift of the 2' hydrogen resonance in the ^{1}H NMR spectrum.

Finally, a number of ant-repellent sesquiterpenoids were isolated from the most nonpolar fractions. These were identified from their physical and spectral characteristics as caryophyllene, caryophyllene epoxide (previously reported as toxic to the leafcutter ant [3]), muurolene [10], and a mixture of cadinene hydrocarbons. β-Sitosterol and *trans*-phytol, an uncommon diterpenoid [11], also were isolated from these fractions. While the crude chloroform extract of these leaves was strongly repellent to leafcutter ants in our bioassays [13], the bulk of the activity was found to reside in this terpenoid fraction.

EXPERIMENTAL

NMR spectra were obtained at 360 MHz using $CDCl_3$ as solvent; chemical shifts are reported in ppm downfield from TMS. Low resolution MS were recorded at 70 eV in/the EI mode; only selected ions are reported here. High resolution MS were obtained at the Midwest Center for Mass Spectrometry, Lincoln NB.

Isolation of compounds. Air dried leaves (*ca* 162 g) were extd successively with $CHCl_3$ and MeOH. Upon bioassay of both exts, the activity was found to reside in the $CHCl_3$ ext. Accordingly, this was concd under red press and the oily residue partitioned between hexane and MeOH–H_2O (1:1). The active hexane fraction was concd to give a thick oil, which was purified by CC over silica gel eluting with hexane–EtOAc or toluene–EtOAc–AcOH. Further purification of the more polar compounds was achieved using either flash column or radial TLC. Final purification of the ant-repellent hydrocarbons was obtained by argentation chromatography on a flash column (3% Ag^+) or with a radial system (2% Ag^+). The following quantities were isolated: piperochromenoic acid (50 mg), piperochromanoic acid (25 mg), piperoic acid (196 mg), 4-hydroxy-5-(*E,E*-farnesyl)benzoic acid (107 mg), 7,4'-dimethoxy-3'-hydroxyflavone (13 mg), *trans*-phytol (92 mg), (−)-γ-muurolene (8 mg), caryophylene oxide (44 mg), caryophylene (32 mg), an isomeric mixture of cadinene hydrocarbons (38 mg), an incompletely characterized sesquiterpene (50 mg), and sitosterol (48 mg).

Piperochromenoic acid (**1**). Brown amorphous solid, $[\alpha]_D^{34}$ + 8 ($CHCl_3$; C 1.0). UV λ_{max}^{MeOH} nm: 238, 283, 305, 318. IR ν_{max} cm^{-1}: 3400, 1730, 1700, 1602, 1300. ^{1}H NMR: δ1.43, 1.55, 1.59, 1.67 (each 3H, *s*), 1.92–2.14 (8H, *m*, 4 × CH_2), 5.05, 5.11 (each 1H, *m*), 5.61 (1H, *d*, *J* = 10 Hz), 6.39 (1H, *d*, *J* = 10 Hz), 6.79 (1H, *d*, *J* = 8.5 Hz), 7.72 (1H, *d*, *J* = 2 Hz), 7.87 (1H, *dd*, *J* = 8.5, 2 Hz). ^{13}C NMR: see Table 1. EIMS *m/z* (rel. int.): 340 ([M]$^+$, 2), 325 ([M − 15]$^+$, 1), 252 (1), 189 (100), 151 (0.2), 115 (3), 91 (43), 81 (3), 77 (1), 69 (15), 55 (2). HR/MS, Found: [M]$^+$ 340.2036 (calcd for $C_{22}H_{28}O_3$: 340.2031).

The Me ester was obtained by dissolving compound **1** (6.5 mg) in MeOH (5 ml) and conc H_2SO_4 (0.5 ml), and heating the resultant soln under reflux for 6 hr. Aq work-up followed by CC (silica gel, 5% EtOAc in hexane), gave the Me ester of compound **1** as an aromatic oil (*ca* 2 mg). ^{1}H NMR: δ1.43, 1.55, 1.58, 1.66 (each 3H, *s*), 3.86 (3H, *s*, OMe), 5.00, 5.09 (each 1H, *m*), 5.61 (1H, *d*, *J* = 10 Hz), 6.39 (1H, *d*, *J* = 10 Hz), 6.77 (1H, *d*, *J* = 8.5 Hz), 7.65 (1H, *d*, *J* = 2.0 Hz), 7.79 (1H, *dd*, *J* = 8.5, 2.1 Hz).

Piperochromanoic acid (**4**). Green aromatic oil, $[\alpha]_D^{34}$ + 3 ($CHCl_3$). UV λ_{max}^{MeOH} nm: 227, 257, 301. ^{1}H NMR: δ1.58, 1.68, 1.71 (each 3H, *s*), 1.89–2.24 (8H, *m*, 4 × CH_2), 3.33 (2H, *br s*), 4.07 (1H, *dd*, *J* = 6.4, 6.2 Hz), 4.81, 4.91 (each 1H, *br s*), 5.14 (1H, *t*, *J* = 6.1 Hz), 5.31 (1H, *t*, *J* = 6.7 Hz), 7.45 (2H, *br s*). ^{13}C NMR (see Table 1). EIMS *mfl z* (rel. int.): 356 ([M]$^+$, 1), 341 ([M − 15]$^+$, 1), 313 ([M − 43]$^+$, 2), 205 (24), 189 (11), 167 (25), 161 (14), 147 (13), 125 (60), 107 (78), 93 (73), 69 (62), 43 (100). HR MS Found: [M]$^+$ 356.1975 (calcd for $C_{22}H_{28}O_4$: 356.1989).

Piperoic acid (**5**). Aromatic oil, UV λ_{max}^{MeOH} nm: 228, 264, 296. IR ν_{max} cm^{-1}: 3383, 2986, 1681, 1442, 1265, 1157. ^{1}H NMR: δ1.58, 1.61, 1.66 (each 3H, *s*), 1.76 (3H, *d*, *J* = 0.6 Hz), 1.87–2.21 (8H, *m*, 4 × CH_2), 3.38 (2H, *d*, *J* = 7.1 Hz), 5.08 (1H, *dt*. *J* = 5.3, 1.3 Hz), 5.15 (1H, *dt*, *J* = 6.8, 1 Hz), 5.31 (1H, *br t*, *J* = 6.8 Hz), 7.51 (2H, *br s*). ^{13}C NMR (see Table 1). EIMS *m/z* (rel. int.): 358 ([M]$^+$, 1.2), 343 ([M − 15]$^+$, 0.1), 191 (7), 168 (14), 167 (17), 129 (10), 92 (6), 91 (18), 79 (12), 77 (13), 69 (100), 53 (10), 41 (24). HRMS Found: [M]$^+$ 358.2151 (calcd. for $C_{22}H_{30}O_4$: 358.2136).

4-Hydroxy-5-(E,E-farnesyl)benzoic acid (**6**). Aromatic oil, UV λ_{max}^{MeOH} nm: 221, 255; (+ NaOH): 218, 250, 277, 286. IR ν_{max} cm^{-1}: 3381, 2935, 1655, 1452, 1025. IR and ^{1}H NMR, identical with lit. values [8]. ^{13}C NMR (see Table 1). EIMS *m/z* (rel. int.): 342 ([M]$^+$, 1), 299 ([M − 43]$^+$, 1), 271 (4), 189 (7.6), 161 (9), 151 (26.7), 136 (12.5), 123 (8.8), 107 (6), 95 (8), 91 (10), 81 (27), 69 (100), 67 (9), 55 (10). HRMS, Found: [M]$^+$ 342.2188 (calcd for $C_{22}H_{30}O_3$: 342.2187).

7,4'-Dimethoxy-3'-hydroxyflavone (**7**). Yellow amorphous solid, ^{1}H NMR: δ4.00, 3.88 (each 3H, *s*, OMe), 6.01 (1H, *s*), 6.37 (1H, *d*, *J* = 2.2 Hz), 6.49 (1H, *d*, *J* = 2.2 Hz), 6.56 (1Hl *s*, 3'-OH), 7.03 (1H, *d*, *J* = 8.4 Hz), 7.33 (1H, *d*, *J* = 1.9 Hz), 7.48 (1H, *dd*, *J* = 8.4, 1.9 Hz), 12.79 (1H, *s*, *J* = 5-OH). EIMS *m/z* (rel. int.): 314 ([M]$^+$, $C_{17}H_{14}O_6$), 285 (24.6), 271 (17), 167 (32), 148 (22), 143 (30), 138 (11), 136 (10), 133 (23), 123 (16), 121 (12), 105 (17), 95 (24), 86 (17), 84 (29).

(−)-γ-Muurolene (**8**). Recovered from hexane–EtOAc as white amorphous solid, was identified by comparison with lit. data (^{1}H NMR [10]): ^{13}C NMR; 15.5 (*q*), 21.7 (*q*), 23.9 (*q*), 25.4 (*t*), 25.9 (*t*), 26.7 (*d*), 30.9 (*t*), 31.6 (*t*), 39.8 (*d*), 43.6 (*d*), 44.8 (*d*), 106.5 (*t*), 124.6 (*d*), 133.8 (*s*), 152.8 (*s*).

trans-*Phytol* (**9**). Green amorphous solid, identical (^{1}H and ^{13}C NMR) with lit. values [11, 12].

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