

PHENYLPROPENE, BENZOIC ACID AND FLAVONOID DERIVATIVES FROM FRUITS OF JAMAICAN *PIPER* SPECIES

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Key Word Index—*Piper aduncum*; *P. hispidum*; Piperaceae; fruits; pseudo-dillapiolide; 4-methoxy-3,5-bis(3'-methyl-2'-butenyl)-benzoic acid; flavonoids.

Abstract—1-Allyl-2,3-(methylenedioxy)-4,5-dimethoxybenzene, 4-methoxy-3,5-bis(3'-methyl-2'-butenyl)-benzoic acid, and the known compounds 5-hydroxy-7-methoxyflavanone and 2,6-dihydroxy-4-methoxydihydrochalcone have been isolated from the fruits of Jamaican *Piper aduncum* and *Piper hispidum*.

INTRODUCTION

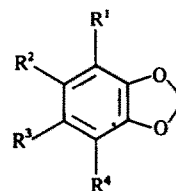
The Piperaceae with its three genera *Piper*, *Pothomorphe* and *Peperomea* are found commonly in Jamaican thickets and limestone area, at an altitude of 17–670 m. The genus *Piper* is widely used in folklore medicine in Latin America and the West Indies [1–6]. In Jamaica, of the eleven *Piper* species known, *Piper aduncum* L. and *Piper hispidum* Sw. are listed as remedies for cold and stomach aches and as insect-repellents [1]. *Piper fadyenii* C.DC., endemic to Jamaica, though not noted for its biological activity, was previously investigated by Burke *et al.* [7] in an attempt to study the chemosystematics of new and old world *Piper*. They reported the isolation, characterization and synthesis of the *E*- and *Z*-isomers of a simple butenolide, fadyenolide. Prior to this, several other lactones isolated from *Piper* have been used as taxonomic indicators [8–11]. In addition to these lactones, terpenes [12, 13], alkaloids [14–18] and flavonoids [19, 20] have also been found in other medicinally important *Piper* species, especially in those native to the Middle and Far East. These discoveries, especially of fadyenolide, 5,6-*Z*- and *E*-butenolide in the endemic *P. fadyenii*, prompted the investigation of other Jamaican *Piper* species. We here report the results of our findings on the fruits of *P. aduncum* and *P. hispidum*.

RESULTS AND DISCUSSION

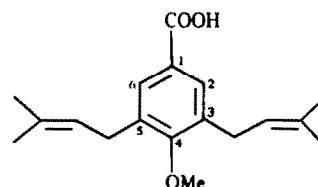
Cold petrol extraction of the dried, milled fruits of *P. aduncum* and *P. hispidum* collected in the hills of St. Andrew, Jamaica, afforded a yellow viscous oil in each case upon removal of the solvent. Evaluation of each crude extract by TLC indicated a strikingly similar profile of compounds. The crude extracts were purified by silica column chromatography, followed by preparative TLC. This gave compounds 1a, 2 and 4. Methanol extraction of the defatted residue gave compound 3. All four com-

pounds were obtained from each plant extract.

Compound 1a, C₁₂H₁₄O₄, a colourless and aromatic oil gave $\lambda_{\text{max}}^{\text{EtOH}}$ at 208 and 285 nm and $\nu_{\text{max}}^{\text{film}}$ at 1625 cm⁻¹. The ¹H NMR spectrum showed signals for two methoxy, one methylenedioxy and one allyl group as well as single aromatic proton. Considering all the spectral evidence,

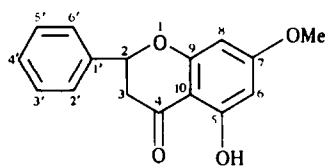


	R ¹	R ²	R ³	R ⁴
1a	OMe	OMe	H	CH ₂ CH=CH ₂
1b	OMe	OMe	CH ₂ CH=CH ₂	H
1c	H	OMe	OMe	CH ₂ CH=CH ₂
1d	OMe	H	OMe	CH ₂ CH=CH ₂
1e	OMe	CH ₂ CH=CH ₂	OMe	H
1f	OMe	H	CH ₂ CH=CH ₂	OMe
1g	OMe	OMe	H	CH=CHMe
1h	OMe	OMe	COOH	H
1i	OMe	OMe	H	COOH
1j	OMe	H	COOH	OMe

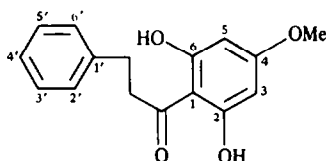


2

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including the ^{13}C NMR, six possible structural forms were evident for compound 1a. The proposed structure (1a) was confirmed by chemical transformations.

The double bond of the allyl group was first isomerized into conjugation with the aryl ring using alcoholic potassium hydroxide. The resulting olefinic bond was then cleaved with osmium tetroxide–sodium periodate in aqueous THF to produce the corresponding carboxylic acid (1i). The melting point ($68\text{--}69^\circ$) of this acid differed considerably from those of the two isomeric acids, dillapiolic acid (1b) (mp $150\text{--}151^\circ$) and apiolic acid (1j) (mp $175\text{--}176^\circ$) [14] which are derivatives of dillapiole (1b) and apiole (1f), respectively [21]. The synthesis of dillapiole and apiole have been reported by Dallacker [22] and the corresponding acids with *ortho* hydrogens fully characterized. This eliminated structures 1b and 1f from consideration. Thus, only four possible isomers, (1a, c, d and e), remained possible structures of this compound.

The structure of the acid was resolved primarily by NMR techniques since several efforts to decarboxylate the acid failed to afford a product with a tetrasubstituted aromatic ring. This latter compound with two aryl protons would have indicated the relative orientation of the proton and the carboxylate group in the acid. The differences in the chemical shifts of the aryl proton in the olefins (1a and 1g) and the corresponding acid (1i) indicated that the aryl proton was in the position *ortho* to the alkenyl side chain. This was confirmed by observing the shift in the position of this aryl proton in the ^1H NMR spectrum of 1i with the addition of a euroshift reagent, tris[3-(heptafluoropropyl)hydroxymethylene]-2-camphorato]europium (III). A mole ratio of 0.77 mole shift reagent to 1.0 mole acid (1i) in CDCl_3 produced a downfield shift of 2.51 ppm in the position of the aryl proton. Other groups experienced shifts of 0.53, 0.73 ($2 \times$ methoxy), 0.31 (methylenedioxy) and 3.30 (acid proton) ppm to lower fields. When the euroshift reagent was checked against 4-(3',3'-dimethyl allyl)-benzoic acid, the *ortho* protons (H-2, H-6) were similarly shifted, the magnitude being significantly larger than that of the *meta* protons (H-3, H-5). The shifts were 2.14 and 0.44 ppm, respectively. Signals for the methylene proton of the

dimethyl allyl group showed a shift comparable to that shown by the methoxy and methylenedioxy groups of the acid 1i. Hence, of the four possible structures remaining, only the acid derived from pseudodillapiole (1a) has a proton *ortho* to the carboxylate group. Our compound is therefore pseudo-dillapiole (1a), which to our knowledge is the first time it has been found as a natural product.

Compound 2, $\text{C}_{18}\text{H}_{24}\text{O}_3$, mp $94\text{--}96^\circ$ was obtained from petroleum. The IR $\nu_{\text{max}}^{\text{KBr}}$ at 2950, 1695 and 1600 cm^{-1} , UV $\lambda_{\text{max}}^{\text{EtOH}}$ at 242 and 208 nm, ^1H NMR and ^{13}C NMR spectra confirmed that this compound was the substituted *p*-methoxybenzoic acid (2). The appearance of the two ring protons as a low field singlet at $\delta 7.79$ established that they are *ortho* to the carboxylic acid. The number of signals in the ^1H and ^{13}C NMR spectra, in particular, highlighted the symmetrical substitution pattern on the benzene ring. The shift in the carbonyl absorption band in forming the methyl ester (1720 cm^{-1}) from the acid (1695 cm^{-1}) and the lack of base shift in the UV spectrum of the latter confirmed that 2 is the carboxylic acid and not the isomeric phenolic ester. Since the onset of our work, this compound was reported to be present in a Colombian *Piper* sp. [23].

The crystals obtained from the methanolic extract was shown to be the pinostrobin (3), $\text{C}_{16}\text{H}_{14}\text{O}_4$, mp $103\text{--}104^\circ$, by comparison of the spectral data [24–26], UV, IR, ^1H and ^{13}C NMR spectra and GC-MS of its silylated derivative. The values for the optical rotation, $[\alpha]_D^{25} - 8^\circ$ (c 1.0; CHCl_3) and the mp of 3 compared with the range of literature values [24–26] and indicated that our isolate differ in enantiomeric purity. To evaluate the latter, the ^1H NMR spectrum of 3 was checked using a chiral shift reagent, (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [27–29]. The chiral shift reagent, 3.93 mole ratio, resolved the C-2 proton into two doublet of doublets for the S and R enantiomers, which had appeared previously as one doublet of doublets at $\delta 5.40$. The integral ratio of these two doublet of doublets at 5.24 and 5.29 ppm, respectively, were 53:47. Since the measured molecular rotation represented by this molecular excess was -8° it is clear that the molecular rotation of the 2S-anomer of 3 should be about -130° .

The biogenetically related dihydrochalcone (4), $\text{C}_{16}\text{H}_{16}\text{O}_4$, mp $164\text{--}165^\circ$ was also obtained from ethyl acetate–petrol, and was characterized by UV, IR, NMR and by MS. In addition, 3 and 4 were correlated by conversion of 3 to 4 by reductive cleavage at C-2 with zinc in acetic acid.

Piper aduncum and *P. hispidum* are easily distinguished by their fruits [30]. Yet, despite the morphological differences, our results show a similar biochemistry. It is interesting that flavanones and chalcones, but no 5- or 6-membered ring lactones, nor piperamides [7–11, 19, 20, 25] found in fruits of several *Piper* species, were detected in the fruits of the two *Piper* species described.

EXPERIMENTAL

General methods. Elemental analysis were in agreement with molecular formulae. Mps are uncorr. ^1H NMR (60, 300 and 400 MHz) and ^{13}C NMR (75.45 MHz): CDCl_3 . MS (70 eV, EI); $[\alpha]_D^{25}$: CHCl_3 ; UV: EtOH, KBr, CHCl_3 or as film. Prep. TLC, TLC and CC were carried out on silica gel using a petrol– Me_2CO solvent system.

Plant material. Fruits of *P. aduncum* and *P. hispidum* were

collected in Newcastle, St. Andrew, Jamaica, and samples are identified and stored in the herbarium at the Institute of Jamaica, Kingston, Jamaica.

Extraction and isolation of compounds 1a, 2, 3 and 4. Dried, milled fruits of *P. aduncum* and *P. hispidum* (150 g each) were separately extracted with cold petrol, affording 16 g each of an oily, yellow, fragrant gum. The crude extract was fractionated on a silica gel column with petrol–Me₂CO mixtures. Extended CC and TLC gave the pure compounds 1a, 2 and 4. The plant residue, after the petrol extraction, was air dried and extracted with cold MeOH. On concn of the extract, compound 3 was crystallized out. It was filtered off and recrystallized from MeOH. The percentage yield of compounds 1a–4, with respect to the dried plant material, were 1.03, 0.70, 1.54 and 0.74 respectively.

Compound 1a. Volatile, aromatic, colourless oil, C₁₂H₁₄O₄ (M_r, 222); UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 208 (3337), 285 (295); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 1625 (olefin); ¹H NMR (CDCl₃, 300 MHz): δ 3.19 (2H, d, J = 9.0 Hz, CH₂ benzylic), 3.76 (3H, s, OMe), 4.01 (3H, s, OMe), 5.05 (2H, m, vinylic CH₂), 5.87 (2H, s, methylenedioxy), 5.92 (1H, m, allylic), 6.30 (1H, s, H-6); ¹³C NMR (CDCl₃, 75.45 MHz): δ 58.08 and 56.33 (both q, OMe), 100.71 (t, OCH₂O), 32.97 (t, benzylic CH₂), 115.43 (t, CH=CH₂), 135.0 (d, CH=CH₂), 104.90 (d, C-6), 113.30 (s, C-1), 146.82 (s, C-2), 140.37 (s, C-5), 137.19 (s, C-3), 131.97 (s, C-4). MS m/z (rel. int.): 222.0891 [M]⁺ (100), 207.0662 [M – Me]⁺ (20.26), 195.0659 [M – CH=CH₂]⁺ (5.57), 191.0712 [M – OMe]⁺ (3.7), 179.0692 [M – Me, CO]⁺ (4.74), 176.0468 [M – OMe, Me]⁺ (3.05), 177.0556 [M – Me, CH₂O]⁺ (34.94).

Double bond isomerization in 1a. Compound 1a (300 mg) refluxed with KOH in EtOH (25%, 10 ml) for 24 hr. The reaction mixture was diluted with H₂O, extracted with EtOAc, dried over anhydrous MgSO₄ and evaporation of the solvent resulted in a viscous oil (1g), purified by TLC; UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 218 (2386), 264 (888), 305 (333); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 1618 (olefin); ¹H NMR (CDCl₃): δ 1.97 (3H, d, J = 6.0 Hz, Me), 3.75 (3H, s, OMe), 4.00 (3H, s, OMe), 5.85 (2H, s, OCH₂O), 6.60 (1H, s, Ar–H), 6.00–6.80 (2H, m, Ar–CH=CH); MS m/z (rel. int.): 222 [M]⁺ (100%).

OsO₄–NaIO₄ oxidation of 1g. The isomerized product (250 mg) was dissolved in THF–H₂O (10:1, 25 ml) and OsO₄ (20 mg) added and stirred for 10 min at room temp. Finely powdered NaIO₄ (600 mg) was added in portions into the stirred mixture (10 min intervals) and left to stir (24 hr). The ppt was filtered off and the filtrate diluted with H₂O (25 ml) and extracted with EtOAc (3 × 15 ml). The organic layer, on evaporation, gave a black residue which was purified by eluting through small column of silica with CHCl₃. The CHCl₃ portion yielded a colourless product (200 mg), recrystallized from petrol as colourless needles (1i), mp 68–69°; UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 220 (10272), 288 (7190), 322 (5157); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1610; ¹H NMR (CDCl₃): δ 3.98 (3H, s, OMe), 4.01 (3H, s, OMe), 6.00 (2H, s, OCH₂O), 7.00 (1H, s, Ar–H), 10.30 (1H, s, exchanged with D₂O, acid); (CDCl₃ + Eu(hfc)₃, 0.77 mole): δ 4.51 (3H, s, OMe), 4.74 (3H, s, OMe), 6.31 (2H, s, OCH₂O), 9.51 (1H, s, Ar–H), 13.60 (1H, s, acid).

Compound 2. Recrystallized from petrol (0–5°), mp 94–94.5°; UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): (6850), 242 (26363); no base shift with KOH–MeOH; IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 1600, 1695 (aromatic acid), 2950 (OH); ¹H NMR (CDCl₃, 300 MHz): δ 1.76 (12H, s, 4 × Me), 3.40 (4H, d, J = 7.0 Hz, 2 × CH₂, benzylic), 3.77 (3H, s, OMe), 5.30 (2H, m, olefinic), 7.79 (2H, s, C-2, C-6), 12.10 (1H, s, exchanged with D₂O, COOH); ¹³C NMR (CDCl₃, 75.45 MHz): δ 17.91 (q, 2 × Me), 25.78 (q, 2 × Me), 60.96 (q, OMe), 28.38 (t, 2 × CH₂), 122.34 (d, 2 × CH=C), 130.27 (d, C-2, C-6), 125.04 (s, C-3, C-5), 135.24 (s, C-1), 133.25 (s, 2 × C=CM₂), 161.20 (s, C-4), 172.20 (s, COOH). MS m/z (rel. int.): 288.1719 [M]⁺ (37.4), 273.1481 [M – Me]⁺ (10.29), 257.1551 [M – OMe]⁺ (15.36), 243.1743 [M – COOH]⁺ (39), 217.0869 (100), 189.0561 (89), 187, 1121 (46.5);

methylation of 2 with CH₂N₂ gave a gummy compound; IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 1720 (ester), 1600; ¹H NMR (CDCl₃): δ 1.74 (12H, s, 4 × Me), 3.40 (4H, d, J = 7.0 Hz, 2 × CH₂), 3.65 (3H, s, 2 × OMe), 7.80 (2H, s, Ar–H), 5.20–5.30 (2H, m, olefin); MS m/z (rel. int.): 302 [M]⁺ (100).

Compound 3. Plate like crystals from MeOH, mp 103–104°, $[\alpha]_D^{25}$ –8° (c1.0, CHCl₃); UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 226 (17102), 287 (18182), 328 (3600); base shift with KOH–MeOH; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3350 (OH), 1660 (C=O), 1600; ¹H NMR (CDCl₃, 300 MHz): δ 2.85 (1H, dd, J = 13.00, 3.30 Hz, C-3 *cis*), 3.10 (1H, dd, J = 13.00, 10.30 Hz, C-3 *trans*), 3.90 (3H, s, OMe), 5.40 (1H, dd, J = 10.30, 3.30 Hz, C-2), 6.03 (2H, d, J = 2.0 Hz, C-6, C-8), 7.40 (5H, m, C-2'–C-6'), 12.00 (1H, s, exchanged with D₂O, phenolic); ¹³C NMR (CDCl₃, 75.45 MHz): δ 55.36 (q, OMe), 43.05 (t, C-3), 78.89 (d, C-2), 162.43, 163.80 and 167.62 (each s, C-5, C-7, C-9), 102.80 (s, C-10), 138.02 (s, C-1'), 94.80 and 93.93 (both d, C-6, C-8), 128.54 and 125.81 (each overlapping d, C-2', C-3', C-4', C-5', C-6') 197.25 (s, C=O); MS m/z (rel. int.): 270.0889 [M]⁺ (100), 269.0828 [M – H]⁺ (39.52), 252.0796 [M – H₂O]⁺ (4.17), 193.0502 [M – C₆H₅]⁺ (96.17), 166.0275 (59.5), 138.0310 (32); GC-MS on the silylated product of 3, m/z : 342 [M + TMS]⁺, 327 [M + (TMS – Me)]⁺, 238 [166 + TMS].

Compound 4. Plate-like crystals from EtOAc–petrol, mp 164–165°; $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 285 (1758), 226 (1430), 208 (2016); bathochromic shift with KOH–MeOH; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3250 (OH), 1660 (C=O), 1600; ¹H NMR (CDCl₃, 300 MHz): δ 3.02 (2H, t, J = 3.0 Hz, CH₂ benzylic), 3.40 (2H, t, J = 3.0 Hz, COCH₂), 3.73 (3H, s, OMe), 5.94 (2H, s, C-3, C-5), 7.26 (5H, s, C-2'–C-6'), 11.80 (1H, s, exchanged with D₂O, phenolic); ¹³C NMR (CDCl₃, 75.45 MHz): δ 54.35 (q, OMe), 45.56 (t, COCH₂), 30.58 (t, benzylic CH₂), 165.71, 163.68 and 157.83 (each s, C-2, C-6, C-4), 141.48 (s, C-1'), 104.95 (s, C-1), 93.29 (d, C-3, C-5), 128.34, 128.20 and 125.65 (each overlapping d, C-2', C-3', C-4', C-5', C-6'), 205.17 (s, C=O); MS m/z (rel. int.): 272.1050 [M]⁺ (17.6), 167.0351 [M – ArCH₂CH₂]⁺ (100), 255.1017 [M – OH]⁺ (4.7), 254.0950 [M – H₂O]⁺ (6.4).

Conversion of 3 to 4. Compound 3 (100 mg) in HOAc (3 ml) was added dropwise to a stirred mixture of Zn dust (300 mg) in water (3 ml). After maintaining the temp. at 20° for 1 hr, the mixture was refluxed for 12 hr. Zinc dust was removed from the reaction mixture and the filtrate worked up in the usual manner to give a solid (60 mg), which upon recrystallization was identical to 4 in every respect.

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