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ANTIFUNGAL BENZOIC ACID DERIVATIVES FROM *PIPER DILATATUM*

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

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Abstract—Six prenylated benzoic acid derivatives and three chalcones have been isolated from the dichloromethane extract of the leaves of *Piper dilatatum* (Piperaceae). Their structures were elucidated by means of mass spectrometry, UV and NMR spectroscopy. Four of the benzoic acid derivatives displayed antifungal properties against *Cladosporium cucumerinum* in direct bioautography on TLC plates. Taboganic acid has been obtained for the first time from plant origin.

INTRODUCTION

In the course of our continuing search for bioactive compounds or lead compounds of plant origin, we are investigating species from the Panamanian flora. One of these plants, *Piper dilatatum* L.C. Rich. (Piperaceae) has been studied since it is used by the Kuna Indians of Panama as a constituent of a mixture of plants applied as a tonic bath for various afflictions. Morever, the Piperaceae family has been shown to be rich in various bioactive chemical classes, such as alkaloids, amides, lignans, flavonoids or lactones [1]. No phytochemical investigation has been reported so far on *P. dilatatum*.

The leaves were extracted successively with dichloromethane and methanol. The nonpolar extract proved to be active against *Cladosporium cucumerinum*, a plant pathogenic fungus, in a bioautographic assay on TLC plates [2]. Features of the spectra obtained by the HPLC-UV analysis of this extract revealed the presence of chalcones and benzoic acid derivatives. Thus, the bioactivity-guided isolation of the antifungal constituents from *P. dilatatum* was undertaken.

RESULTS AND DISCUSSION

The antifungal dichloromethane extract was fractionated by silica gel column chromatography, yielding fourteen fractions (A–N), eight of which showed inhibitory reactions in the TLC bioassay with *C. cucumerinum*.

Compounds **1** and **2** were isolated from fractions M and D, respectively, after purification on Sephadex LH 20 gel. They displayed identical features in their ^{1}H and ^{13}C NMR spectra except for the absence of a signal corresponding to a methyl ester group in compound **1** (δ 52.0 ppm). These observations suggested that compound **2** was the methyl ester of an acid **1**. Their ^{13}C NMR spectra displayed signals for six aromatic carbons, including three-CH forming *ortho-ortho* and *ortho-meta* coupling systems (δ 136.0, 132.0 and 117.9 ppm). Signals due to the carbonyl function of an ester group at δ 166.9 ppm (**2**) and an acid at δ 166.6 ppm (**1**) were also present. The side chain of both compounds consisted of one ketonic carbon (δ 194.4 ppm), one methylene group (δ 117.9 ppm), one unsaturated quaternary carbon (δ 159.3 ppm) and two terminal methyl groups (δ 27.8 ppm and 21.3 ppm). This side chain proved to be in an open form since a signal for the chelated proton of the phenolic group could be observed at low field in the ^{1}H NMR spectrum (δ 12.5 ppm). The structure of the side chain of both **1** and **2** was therefore elucidated as 1-oxo-3-methyl-2-butenyl. These data were confirmed by EI- and D/CI-MS measurements. Compound **2** was identified as taboganic acid methyl ester and has already been isolated from *Piper taboganum* [3]. Compound **1** was the genuine acid of **2** and was named taboganic acid. This acid is reported here for the first time as a natural product. It was the major

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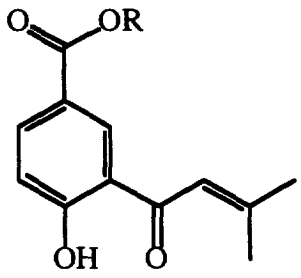
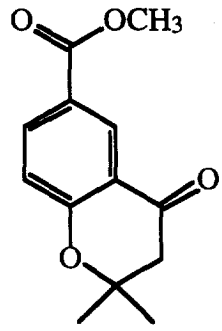


1 R=H
2 R=CH_3



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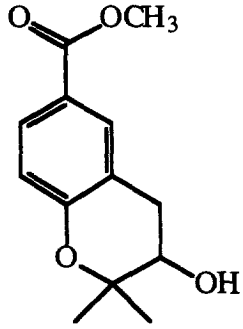

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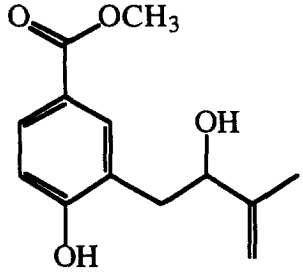

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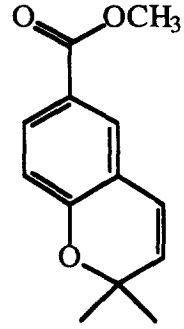

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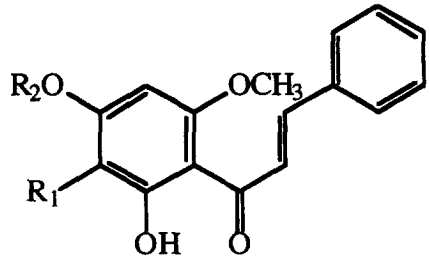

7 R_1=H, R_2=CH_3
8 R_1=H, R_2=H
9 R_1=OCH_3, R_2=CH_3

constituent of the dichloromethane extract and not of artefactual nature since it was detected in the extract before fractionation was undertaken. Compounds **3** and **4** were found to be closely-related derivatives of **1**. The structure of **3** was established from NMR, EI- and D/CI-MS data as the methyl ester of 2,2-dimethyl-6-carboxychroman-4-one, previously isolated from *Piper taboganum* [3].

Comparison with literature data [4, 5] led to the identification of **4** as the methyl ester of 2,2-dimethyl-3-hydroxy-6-carboxychroman. This compound is known from *Eriodictyon sessilifolium* (Hydrophyllaceae) [4] and has also been prepared as a derivative from constituents of *Anodendron affine* (Apocynaceae) [5].

Two additional prenylated benzoic acid derivatives (**5** and **6**) were isolated from fractions C and I respectively. Their structures were elucidated by comparison of spectroscopic data with those of the above described products and with the literature [6]. They were identified as the methyl ester of 2,2-dimethyl-6-carboxychromene (**6**) and the methyl ester of 4-hydroxy-3-(2′-hydroxy-3′-methyl-3′-butenyl)-benzoic acid (**5**). Both compounds have been previously characterised from *Piper hostmannianum* [6].

Three known chalcone derivatives were also isolated and identified by means of their spectral data, namely flavokawain B (**7**), cardamonin or alpinetin chalcone (**8**) and 2′-hydroxy-3′,4′,6′-trimethoxychalcone (**9**). Compound **7** was first obtained

from kawa (*Piper methysticum*) [7]. Alpinetin chalcone has been described in seeds of *Alpinia speciosa* (Zingiberaceae) [8], and in various *Piper* species [1, 9], while chalcone **9** was described first as a constituent from the stem of *Popowia cauliflora* (Annonaceae) [10] and was then also reported from *Piper hispidum* [11].

Compounds **1** to **4** displayed antifungal activity against the plant pathogenic fungus *Cladosporium cucumerinum* in the bioautographic test on TLC plates. The most active product was **3** with only 1 μg inhibiting fungal growth on the plate. This amount was comparable to the minimum quantities in the same assay of miconazole (1 μg) and propiconazole (0.1 μg), two commercially available reference antifungal compounds. Compound **4** was active when 3 μg was deposited on the TLC plate, while 5 μg were required to detect the activity of taboganic acid (**1**) and its methyl ester (**2**). These properties were confirmed in a dilution assay for compounds **2** and **3** by measurement of their minimal inhibitory concentrations (MIC). The MIC values of compounds **3** and **2** were determined as 40 μg/ml and 60 μg/ml, respectively. The values obtained for the reference standards were 1 μg/ml (propiconazole) and 10 μg/ml (miconazole). Though a little lower, the activities of **2** and **3** were still comparable with the effect of the reference antifungal drugs. Taboganic acid (**1**) was not active in the dilution assay, while compound **4** was not tested, due to insufficient availability of product.

EXPERIMENTAL

General

Mps: uncorr., measured on a Mettler FP 80/82 hot-stage apparatus. MS: Finnigan MAT TSQ. UV spectra were recorded in MeOH. ^{1}H and ^{13}C NMR spectra were measured at 200.06 and 50.31 MHz, respectively, in DMSO-d_6 or $CHCl_3$. TMS was used as int. stand. 2D experiments were performed on a Varian INOVA 500 instrument.

Plant material

Aerial parts of *Piper dilatatum* were collected in May 1995 at Rio Diablo, C. Kuna Ayala, Province of San Blas, Panama. The plant material was identified by Dr. R. Calleja, University of Antioquia, Colombia. A voucher sample (FLORPAN 2131) is deposited at the Herbarium of the University of Panama.

Extraction and isolation

Dried powdered aerial parts (200 g) were successively extracted at room temp. with CH_2Cl_2 and MeOH. The CH_2Cl_2 extract (5 g) was submitted to silica gel CC with a gradient of petroleum ether-EtOAc (8:1) to pure EtOAc, yielding 14 frs (A–N). Cpd **1** (60 mg) was isolated from fr M in two separation steps: gel filtration (LH 20, $CHCl_3$-MeOH, 1:1) followed by vacuum liquid chromatography on RP-18 (MeOH-H_2O, 68:32). Cpds **2** (50 mg) and **3** (60 mg), from frs D and F, respectively, after gel filtration on Sephadex LH 20, were purified by crystallization in *n*-hexane-EtOAc. The benzoic acid derivatives **4** (3 mg) and **6** (70 mg), together with chalcones **8** (50 mg) and **9** (30 mg) were obtained from fr J after gel filtration and silica gel CC (*n*-hexane-EtOAc, 2:1). 5 mg of **5** were purified from fr C (gel filtration). The chalcone **7** (3 mg) was isolated from fr E by gel filtration on Sephadex LH 20.

3-(1′-Oxo-3′-methyl-2′-butenyl)-4-hydroxy-benzoic acid (*taboganic acid*) (**1**). Yellow powder. Mp 170–174°C. UV λ_{max}^{MeOH} nm (log ε): 232 (3.7), 321 (3.0). EIMS 70 eV *m/z* (rel. int. %): 220 $[M]^+$ (15), 205 (100), 165 (33). ^{1}H NMR (200.06 MHz, DMSO-d_6): δ 12.5 (1H, *br s*, OH-4), 8.4 (1H, *d*, *J* = 1.8 Hz, H-2), 8.05 (1H, *dd*, *J* = 1.9, 8.5 Hz, H-6), 7.05 (1H, *d*, *J* = 8.5 Hz, H-5), 7.0 (1H, *br s*, H-2′), 2.2[a] (3H, *s*, H-4′), 2.07[a] (3H, *s*, H-5′). ^{13}C NMR (50.3 MHz, DMSO-d_6): δ 194.4 (C-1′), 166.6 (C-7), 164.4 (C-4), 159.3 (C-3′), 136.0 (C-6), 132.0 (C-2), 121.5 (C-3), 120.6 (C-1), 120.6 (C-5), 117.9 (C-2′), 27.8[b] (C-4′), 21.3[b] (C-5′). [a,b]Assignments interchangeable.

Methyl 3-(1′-oxo-3′-methyl-2′-butenyl)-4-hydroxy-benzoate (*methyl taboganate*) (**2**). Yellow prisms. Mp 96–97°C (lit. 91–95°C [3]). UV λ_{max}^{MeOH} nm (log ε): 232 (4.0), 261 (3.9), 328 (3.4). EIMS 70 eV *m/z* (rel. int. %): 234 $[M]^+$ (8), 219 (100), 179 (10). ^{1}H and ^{13}C NMR measurements agree with previously reported data [3].

2,2-Dimethyl-6-carboxychroman-4-one methyl ester (**3**) White crystals. Mp 101.5°C (lit. 92°C [12]). UV λ_{max}^{MeOH} nm (log ε): 232 (3.9), 320 (3.12). EIMS 70 eV *m/z* (rel. int. %): 234 $[M]^+$ (44), 219 (100), 179 (54). ^{1}H and ^{13}C NMR data in agreement with lit. [3, 12].

2,2-Dimethyl-3-hydroxy-6-carboxychromane methyl ester (**4**). White amorphous powder. UV λ_{max}^{MeOH} nm (log ε): 206 (3.8), 261 (3.7). EIMS 70 eV *m/z* (rel. int. %): 236 $[M]^+$ (65), 219 (12), 195 (16), 165 (100), 107 (27). ^{1}H and ^{13}C NMR data in agreement with lit. [5] and [4], respectively.

Methyl 3-(2′-hydroxy-3′-methyl-3′-butenyl)-4-hydroxy-benzoate (**5**). White powder. Mp 124–126°C (lit. 120°C [6]). UV λ_{max}^{MeOH} nm (log ε): 217 (3.7), 251 (3.6). EIMS 70 eV *m/z* (rel. int. %): 236 $[M]^+$ (12), 203 (8), 166 (100). ^{1}H and ^{13}C NMR data in agreement with lit. [6].

2,2-Dimethyl-6-carboxychromene methyl ester (**6**). Yellow oil. UV λ_{max}^{MeOH} nm (log ε): 237 (2.6). EIMS 70 eV *m/z* (rel. int. %): 219 $[M + H]^+$ (100), 203 (64), 179 (52), 162 (34). ^{1}H and ^{13}C NMR as reported [6].

2′-Hydroxy-4′,6′-dimethoxy-chalcone (flavokawin) (**7**). Yellow powder. Mp 83–84°C (lit. 91.5–92°C [13]). UV λ_{max}^{MeOH} nm (log ε): 209 (4.0), 339 (3.9). EIMS 70 eV *m/z* (rel. int. %): 284 $[M]^+$ (100), 207 (80), 180 (50). ^{1}H and ^{13}C NMR data as reported [13].

2′,4′-Dihydroxy-6′-methoxy-chalcone (*alpinetin chalcone*) (**8**). Yellow powder. Mp 193–196°C (lit. 195–196°C [13]). UV λ_{max}^{MeOH} nm (log ε): 205 (4.1), 344

(4.0). EIMS 70 eV *m/z* (rel. int. %): 270 $[M]^+$ (75), 193 (100), 167 (40). ^1H and ^{13}C NMR data as in lit. [13].

2′-Hydroxy-3′,4′,6′-trimethoxy-chalcone (**9**). Orange powder. Mp 129–132°C (lit. 136°C [10], 128–130°C [11]). UV λ_{max}^{MeOH} nm (logϵ): 211 (3.9), 324 (3.9). EIMS 70 eV *m/z* (rel. int. %): 314 $[M]^+$ (100), 237 (24), 210 (28), 195 (47), 197 (90). ^1H and ^{13}C NMR data as reported [10].

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