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PHENYLPROPANE DERIVATIVES FROM ROOTS OF *COSMOS CAUDATUS*

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Key Word Index— *Cosmos caudatus*; Asteraceae; roots; phenylpropanoids; antifungal activity.

Abstract— One hydroxyeugenol and five coniferyl alcohol derivatives have been isolated from the roots of *Cosmos caudatus*. Structures of the isolated compounds were established on the basis of spectral data. *Z*-coniferyl alcohol-3′-acetyl-4-*iso*butyrate and 1′,2′-dihydroxy-coniferyl alcohol-3′-*iso*butyryl-4-*iso*butyrate are new natural compounds. The other compounds, 1′-acetoxy-eugenol-4-*iso*butyrate, 1′,2′-epoxy-*Z*-coniferyl alcohol-3′-(2-methylbutyryl)-4-*iso*butyrate, 1′,2′-epoxy-*Z*-coniferyl alcohol-3′-acetyl-4-*iso*butyrate and 1′,2′-epoxy-*Z*-coniferyl alcohol-3′-*iso*butyryl-4-isobutyrate, have been described from species of the same tribe (Heliantheae), but their antifungal properties are reported here for the first time.

INTRODUCTION

As part of our ongoing search for biologically active principles from tropical plants, we have studied *Cosmos caudatus*. This species is a native of tropical America and is naturalized on Java, where it is often cultivated as an ornamental. Until now, no phytochemical investigations have been carried out on the genus *Cosmos*. However, species belonging to the same tribe as *Cosmos* (Heliantheae) have already been investigated [1–9]; sesquiterpene lactones, polyacetylenes, flavonoids and some phenylpropanoids with unusual structures have been isolated. The latter compounds showed a significant anti-ulcer activity in rats and anti-tumour activity against Sarcoma 180 ascites in mice [10].

A dichloromethane root extract of *C. caudatus* showed antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* in a bioautographic assay on TLC [11, 12]. HPLC analysis of the lipophilic extract, using diode-array detection, showed the presence of compounds with UV spectra typical of phenylpropanoids (maximum 274 nm). Activity-guided fractionation afforded six phenylpropane derivatives; we report, herein, the isolation and structural determination of these compounds.

RESULTS AND DISCUSSION

Fractionation of the dichloromethane root extract by open column chromatography on silica gel, followed by low-pressure LC and semi-prep. HPLC on RP-18 columns afforded **1**–**6** (see Experimental). Four of the isolated compounds were identified as **1**, **2**, **4** and **5**, already described in species belonging to the genera, *Coreopsis* and *Bidens* [2–9]. Identification was carried out by EI mass, ^{1}H and ^{13}C NMR spectroscopy, with the aid of NOE measurements. The results obtained were in accordance with literature data [9].

The ^{1}H NMR spectra of **1**, **3** and **4** exhibited some common signals, an aromatic methoxyl (δ3.8), a methyl at δ2.1 characteristic of an acetyl group and signals belonging to an *iso*butyryl group. In the aromatic region, the spectrum of **3** exhibited signals of an *ortho*-coupled proton at δ7.0 and a two-proton multiplet at δ6.8. A spin-system composed of a broad doublet (J = 11.7 Hz) at δ6.6 attributable to an olefinic proton, a double triplet (1 H, J = 11.7 and 6.7 Hz) at δ5.7 and a double doublet at δ4.8 (2 H, J = 6.7 and 1.5 Hz) suggested that **3** was an ester of *Z*-coniferyl alcohol. The EI mass spectrum exhibited a $[M]^+$ at m/z 292. As for **1**, the presence of fragments at m/z 222 (loss of dimethylketene from the *iso*butyryloxy moiety) and 233 (loss of an acetic acid radical), together with the lack of the fragment at m/z 206 ($[M - 86]^+$, loss of *iso*butyryloxy radical) indicated that the *iso*butyryloxy group was directly attached to the aromatic ring, while the acetoxy group was connected to the olefinic side-chain. The position of the *iso*butyryloxy moiety on the aromatic ring was determined with the aid of NOE measurements [13]. Presaturation of the methoxyl signal (δ3.8) resulted in the enhancement of a signal of a *meta*-coupled proton (H-6) at δ6.8. Thus, the structure of **3** was established as 3′-*O*-acetyl-4-*O*-*iso*butyryl-*Z*-coniferyl alcohol.

The EI mass spectrum of **6** exhibited a weak $[M]^+$ at m/z 354, together with an abundant $[M - 17]^+$ signal,

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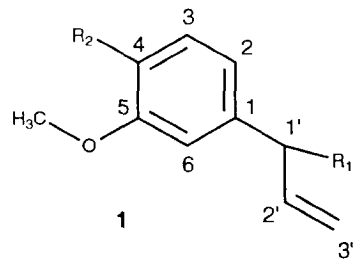

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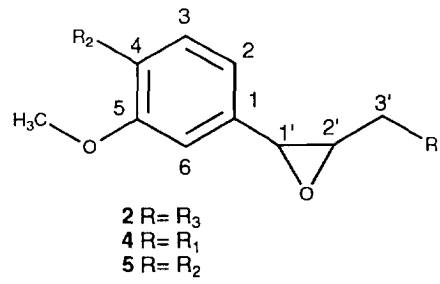

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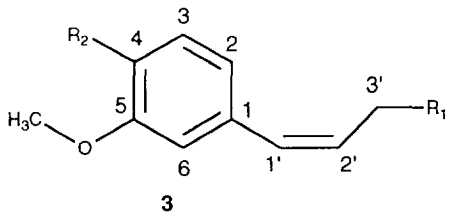
4 R= R_1
5 R= R_2



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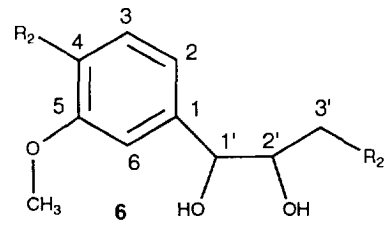

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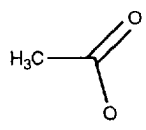
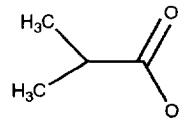


R_1



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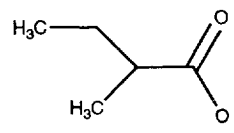

R_3

suggesting the loss of an hydroxyl group. The CI mass spectrum showed an adduct ion $[M + NH_4]^+$ at *m/z* 372, confirming the M_r of **6**. The ^1H NMR of **6** exhibited some common signals with those of **5**: a singlet due to an aromatic methoxyl (δ3.8) and signals belonging to two *iso*butyryl groups. A double-doublet due to an *ortho*, *meta*-coupled aromatic proton (δ6,6), together with a multiplet due to two protons at δ7.0 indicated the presence of asymmetric substitution of the aromatic ring. Signals of H-1′ and H-2′ had the same multiplicity as those of **5**, but they were shifted down-field by *ca* 0.5 ppm, with a coupling constant of 6.3 Hz, suggesting that the epoxy ring was open. The ^{13}C NMR spectrum showed two signals belonging to two hydroxylated CH groups at δ74.0 and 74.1. These data confirmed that **6** was 1′,2′-dihydroxy-4,3′-di-*O*-*iso*butyrylconiferyl alcohol.

The antifungal activities of **1**–**6** against *Cladosporium cucumerinum* and *Candida albicans* were determined in a TLC bioassay [11, 12]. Compounds **1** and **4** were antifungal against *C. cucumerinum* at 0.1 μg, **2**, **3** and **5** at 1, 5 and 0.5 μg, respectively, while **6** was devoid of activity. In the same assay, the synthetic fungicide, propiconazole, was active at 0.01 μg. Only **2**, **4** and **5** were active against *C. albicans* at 5 μg. The amount of the reference compound, miconazole, required to inhibit the growth of *C. albicans* on TLC plates was 1 μg.

One 1′-hydroxyeugenol and five coniferyl alcohol derivatives have been isolated from the lipophilic root extract of *C. caudatus*. In spite of their rather simple structure, **3** and **6** are new natural products. The co-occurrence of eugenol and *Z*-coniferyl alcohol derivatives in the Asteraceae and Umbelliferae has been used as a marker to show the close relationship between the families [14]. However, structural revision of some of the compounds isolated from *Pimpinella* species [15, 16] prevented the use of these constituents as a basis for the discussion of the chemotaxonomic relationship between the two families. Until now, eugenol and *Z*-coniferyl alcohol derivatives have been isolated only in *Coreopsis*, *Bidens* and *Cosmos* species (Asteraceae) [2–9]. Since these species belong to the tribe Heliantheae, these compounds could be useful chemotaxonomic markers of this tribe.

Although phenylpropane derivatives are well known antifungal compounds [17], this is the first report on the activities of **1**–**5**. Only **2**, **4** and **5** showed activity against *C. albicans* suggesting that the epoxy moiety is an import-

ant structural element. On the contrary, for activity against *C. cucumerinum* it is not possible to make any comment on structure–activity relationships.

EXPERIMENTAL

General. Mps: uncorr. TLC was carried out on silica gel precoated AI sheets (Merck). The solvent systems employed were petrol–EtOAc (1:1) (system A) and CH_2Cl_2–MeOH (97:3) (system B). For open CC, silica gel (40–63 μm) was used. UV spectra were recorded in MeOH. HPLC was performed on an instrument equipped with a photodiode-array detector. Purity of compounds was checked on Nova-Pak RP-18 columns (4 μm, 3.9 × 150 mm i.d., Waters) at a flow rate of 1 ml min^{-1}. LPLC was carried out on a pre-packed Lobar column (LiChroprep, RP-18, 40–63 μm, Merck) at a flow rate of 2 ml min^{-1}. Semi prep. HPLC was performed on LiChrosorb RP-18 columns (7 μm, 250 × 16 mm i.d., Knauer) at a flow rate of 10 ml min^{-1}. MS were recorded using a triple-stage quadrupole instrument. D/CIMS: positive ion mode, NH_3 as reactant gas. ^1H and ^{13}C NMR were measured at 200 and 50 MHz in $CDCl_3$. NOE measurements were carried out according to ref. [13].

Plant material. Cosmos caudatus was collected in August 1993 near Purwodadi on the island of Java, Indonesia. The species was identified by the Institute Herbarium Bogoriensis, Bogor, Indonesia. A voucher specimen is deposited at the Institute Herbarium Bogoriensis, Bogor, Indonesia.

Extraction and isolation. Dried and ground roots (500 g) were successively extracted at room temp. with CH_2Cl_2 and MeOH. The CH_2Cl_2 extract (4 g) was submitted to CC on silica gel (40–63 μm) using step gradient elution (petrol–EtOAc, 9:1, 6:1, 3:1, 1:1, 0:1); 18 frs were collected. Semi prep. HPLC of fr. 9 (69 mg) and 10 (73 mg) on RP-18 using a step gradient (MeOH–H_2O, 13:7 for 10 min, then from 13:7 to 4:1 in 10 min) afforded **1** (30 mg), **2** (9 mg) and **3** (2.5 mg). Compound **4** (30 mg) was obtained from fr. 13 (390 mg) by Sephadex LH-20 CC with MeOH–$CHCl_3$ (1:1), followed by semi-prep HPLC on RP-18 with MeOH–H_2O (13:7). Frs 12 (120 mg) and 11 (819 mg) were washed with MeOH. The soluble portion of fr. 12 afforded **5** (40 mg) after sepn by semi-prep HPLC on RP-18 with MeOH–H_2O (13:7). LPLC on RP-18 of the soluble portion of fr. 11 using step gradient elution (MeOH–H_2O, 13:7, 7:3, 4:1) afforded **5** (40 mg) and **2** (15 mg). Compound **6** (13 mg) was obtained from fr. 15 (280 mg) by Sephadex LH-20 CC with MeOH–$CHCl_3$ (1:1), followed by semi-prep HPLC on RP-18 with MeOH–H_2O (11:9).

1′-Acetoxy-4-O-isobutyryleugenol (**1**). Dark yellow oil. TLC (system A): R_f = 0.57, TLC (system B): R_f = 0.75. UV λ_{max}^{MeOH} nm (log ε): 219 (3.85), 272 (3.37). $[\alpha]_D^{25}$ − 80.9 ($CHCl_3$; *c* 0.75). D/CIMS *m/z* (rel. int.): 310 $[M + NH_4]^+$ (84), 233 (100). EIMS, ^1H NMR and ^{13}C NMR as ref. [9].

1′,2′-Epoxy-4-O-isobutyryl-3′-O-(2-methylbutyryl)-Z-coniferyl alcohol (**2**). Dark yellow oil. TLC (system A): R_f = 0.59, TLC (system B): R_f = 0.77. UV λ_{max}^{MeOH} nm (log ε): 221 (3.83), 272 (3.31). $[\alpha]_D^{25}$ − 25.9 ($CHCl_3$; *c*0.92). D/CIMS *m/z* (rel. int.): 368 $[M + NH_4]^+$ (100), 351 $[M + H]^+$ (30). EIMS, ^1H NMR and ^{13}C NMR as ref. [9].

3′-O-Acetyl-4-O-isobutyryl-Z-coniferylalcohol (**3**). Oil. TLC (system A); R_f = 0.57, TLC (system **B**): R_f = 0.75. UV λ_{max}^{MeOH} nm (log ε): 245 (3.34), 282 (2.78). EIMS *m/z* (rel. int.): 292 $[M]^+$ (22), 233 (7) 222 (100), 179 (60), 131 (21). D/CIMS *m/z* (rel. int.): 310 $[M + NH_4]^+$ (100). ^1H NMR [200 MHz, $CDCl_3$]: δ7(1H, *d*, *J* = 8 Hz, H-3), 6.8 (2 H, *m*, H-2 and H-6), 6.6 (1H, *br d*, *J* = 11.7 Hz, H-1′), 5.8 (1H, *dt*, *J* = 6.7, 11.7 Hz, H-2′), 4.8 (2H, *dd*, *J* = 6.7, 1.5 Hz, H-3′), 3.8 (3H, *s*, MeO-5), 2.8 (1H, *st*, *J* = 7 Hz, H-2″), 2.1 (3H, *s*, Me-2‴), 1.3 (6H, *d*, *J* = 7 Hz, Me-3″ and Me-4″). ^{13}C NMR [50 MHz, $CDCl_3$]: δ175.2 (C-1″), 171.1 (C-1‴), 150.9 (C-5), 139.4 (C-4), 134.7 (C-1), 132.7 (C-1′), 125.9 (C-2′), 122.6 (C-3), 121.2 (C-2), 112.8 (C-6), 61.3 (C-3′), 55.9 (MeO-5), 34.0 (C-2″), 21.0 (C-2‴), 19.0 (C-3″ and C-4″).

1,2′-Epoxy-3′-O-acetyl-4-O-isobutyryl-Z-coniferyl alcohol (**4**). Dark yellow oil. TLC (system A): R_f = 0.44, TLC (system B): R_f = 0.71. UV λ_{max}^{MeOH} nm (log ε): 220 (3.9), 272 (3.38). $[\alpha]_D^{25}$ − 33.7 ($CHCl_3$; *c* 1.63). D/CIMS *m/z* (rel. int.): 326 $[M + NH_4]^+$ (100), 309 $[M + H]^+$ (20). EIMS, ^1H NMR and ^{13}C NMR as ref. [9].

1′,2′-Epoxy-3′,4-di-O-isobutyryl-Z-coniferyl alcohol (**5**). Dark yellow oil. TLC (system A): R_f = 0.54, TLC (system B): R_f = 0.74. UV λ_{max}^{MeOH} nm (log ε): 221 (3.9), 271 (3.36). $[\alpha]_D^{25}$ − 33.7 ($CHCl_3$; *c*2.24). D/CIMS *m/z* (rel. int.): 354 $[M + NH_4]^+$ (100), 337 $[M + H]^+$ (30). EIMS, ^1H NMR and ^{13}C NMR as ref. [9].

1′,2′-Dihydroxy-3′,4-O-isobutyrylconiferyl alcohol (**6**). Oil. TLC (system A): R_f = 0.22, TLC (system B): R_f = 0.29. UV λ_{max}^{MeOH} nm (log ε): 245 (3.34), 282 (2.78). $[\alpha]_D^{25}$ − 1.31 ($CHCl_3$; *c* 0.91). EIMS *m/z* (rel. int.): 354 $[M]^+$ (10), 337 (79), 249 (8), 223 (42), 153 (100), 71 (40). D/CIMS *m/z* (rel. int.): 372 $[M + NH_4]^+$ (100). ^1H NMR [200 MHz, $CDCl_3$]: δ7.0 (2H, *m*, H-3 and H-6), 6.9 (1H, *dd*, *J* = 8, 1.6 Hz, H-2), 4.6 (1H, *d*, *J* = 6.3 Hz, H-1′), 4.15 (1H, *dd*, *J* = 3.6, 11.4 Hz, H-3′a), 4 (1H, *dd*, *J* = 5.9, 11.4 Hz, H-3′b), 3.8 (1H, *m*, H-2′), 3.8 (3H, *s*, MeO-5), 2.8 (1H, *st*, *J* = 7 Hz, H-2″), 2.6 (1H, *st*, *J* = 7 Hz, H-2‴), 1.3 (6H, *d*, *J* = 7 Hz, Me-3″ and Me-4″), 1.3 (6H, *d*, *J* = 7 Hz, Me-3‴ and Me-4‴). ^{13}C NMR [50 MHz, $CDCl_3$]: δ177.5 (C-1″), 175.3 (C-1‴), 151.3 (C-5), 139.8 (C-4), 138.8 (C-1), 122.8 (C-3), 118.8 (C-2), 110.5 (C-6), 74.1 (C-1′), 74.0 (C-2′), 65.0 (C-3′), 55.9 (MeO-5), 34.0 (C-2″, C-2‴), 19 (C-3″, C-4″, C-3‴ and C-4‴).

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