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A PHLORACETOPHENONE GLUCOSIDE WITH CHOLERETIC ACTIVITY FROM *CURCUMA COMOSA*

APICHART SUKSAMRARN,*† SALINEE EIAMONG,† PAWINEE PIYACHATURAWAT‡ and LINDSAY T. BYRNE§

†Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand; ‡Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand; §Department of Organic Chemistry, University of Western Australia, Nedlands, W.A. 6907, Australia

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Key Word Index—*Curcuma comosa*; Zingiberaceae; diarylheptanoids; phloracetophenone glucoside; 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl)acetophenone; choleretic activity.

Abstract—Three known diarylheptanoids, 1,7-diphenyl-5-hydroxy-(1*E*)-1-heptene, 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1*E*)-1-heptene and 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene, were isolated from the ethyl acetate extract of *Curcuma comosa* rhizomes. A phloracetophenone glucoside, 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl)acetophenone, was isolated from the ethyl acetate and *n*-butanol extracts. This compound exhibited choleretic activity in rats.

INTRODUCTION

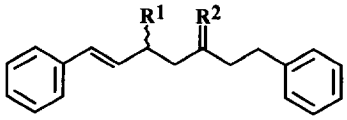
The rhizome of *Curcuma comosa* Roxb. has been used extensively in indigenous medicine in Thailand as an anti-inflammatory agent. It has also been widely used as an aromatic stomachic and also for the treatment of postpartum uterine bleeding and peri-menopausal bleeding. On the basis of local use, we recently found that the hexane extract of this plant exhibited a uterotrophic effect [1] and possessed oestrogenic activity [2], whereas the ethyl acetate and butanol extracts exhibited choleretic effect [3]. In an earlier study, the methanolic extract of its rhizomes was shown to have nematocidal activity against *Caenorhabditis elegans*, and the diarylheptanoids **1–5** were isolated from the less polar fractions of the extract [4]. In this paper, we report on the isolation and identification of three known diarylheptanoids (**3** [4], **6** and **7** [5]) and a new phloracetophenone glucoside (**8**) from the ethyl acetate and butanol extracts of this plant. The choleretic activity of compound **8** was evaluated.

RESULTS AND DISCUSSION

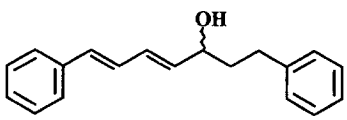
Repeated column chromatography of the EtOAc extract of *C. comosa* rhizomes resulted in the isolation of three diarylheptanoids. The first compound was a non-phenolic diarylheptanoid, the structure of which was identical (^{1}H NMR and electron impact (EI)-MS) to that of 1,7-diphenyl-5-hydroxy-(1*E*)-1-heptene (**3**) isolated previously from the non-polar fraction of the *C. comosa* extract [4]. The second and third compounds were identical (TLC, IR and ^{1}H NMR) to the phenolic diarylheptanoids 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1*E*)-1-heptene (**6**) and 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene (**7**) isolated from the polar fraction of *Curcuma xanthorrhiza* [5].

The most polar component of the EtOAc extract was elucidated as 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl)acetophenone (**8**) on the basis of spectroscopic data. This compound was also isolated from the butanol extract. The elemental analysis and EI-mass spectrum established a molecular formula of $C_{14}H_{18}O_9$. The IR absorption band at 3398 cm^{-1} indicated the presence of hydroxyl groups, and the broad, intense absorption band at 1625 cm^{-1} was indicative of a conjugated keto group. The unusually low IR absorption frequency of the latter functional group suggested intramolecular hydrogen bonding with a hydroxyl group. The signal of a quaternary carbon at δ 204.7 in the ^{13}C NMR spectrum confirmed the presence of a keto function in the molecule. The ^{1}H NMR spectrum revealed the presence of two aromatic protons δ 5.93 *d*, J = 2.2 Hz; 6.17, *d*, J = 2.2 Hz). The coupling-constant value showed these two protons to be *meta* to each other. A three-proton singlet at δ 2.68 accounted for a methyl group attached to an aromatic keto group. The rest of the ^{1}H NMR spectrum clearly

*Author to whom correspondence should be addressed.



1 R^1 = H, R^2 = H, OAc
2 R^1 = H, R^2 = O
3 R^1 = H, R^2 = H, OH
4 R^1 = OH, R^2 = O



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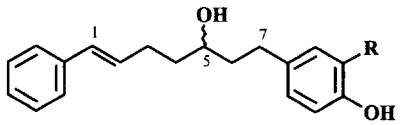
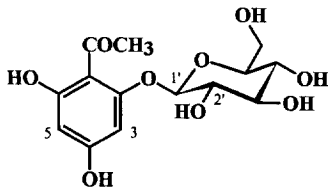


6 R = H
7 R = OH



8

revealed the presence of a glucose moiety in **8**. Thus a one-proton doublet at δ 5.01 (J = 7.4 Hz) could be assigned to the anomeric proton of a β-D-glucoside (i.e. H-1′). The signals at δ 3.71 (*dd*, J = 12.1, 4.9 Hz, 1H), 3.90 (*dd*, J = 12.1, 1.8 Hz, 1H) and 3.37–3.55 (*m*, 4H) accounted for another six protons of the glucose moiety. The alternative possible structure, 2,6-dihydroxy-4-*O*-(β-D-glucopyranosyl)acetophenone, was ruled out, since the symmetrical structure of the latter compound would give only four aromatic carbon atoms in the ^{13}C NMR spectrum. In fact, the spectrum indicated that C-2 and C-6 were non-equivalent, i.e. signals at δ 166.2 and 162.6, respectively. The C-3 and C-5 signals were also non-equivalent, i.e. signals at δ 101.9 and 95.3, respectively. From the above spectroscopic and analytical data, the compound was thus concluded to be 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl)acetophenone (**8**). This is the first report of a substituted acetophenone in *Curcuma* species.

It should be noted that a phloracetophenone-2-glucoside (melting point 201.5–203°), presumably compound **8**, has been synthesized previously, but no spectroscopic data were reported [6].

Biological activity

The phloracetophenone glucoside **8** effectively exhibited choleretic activity in rats, and the activity was dose related.

EXPERIMENTAL

General. Mp: uncorr. Microanalysis was performed by the Scientific and Technological Research Equipment Centre, Chulalongkorn University. ^{1}H and ^{13}C NMR: 300 and 75.5 MHz, respectively. CC and TLC: Merck silica gel 60 (> 230 mesh) and precoated silica gel 60 F_{254} plates, with either $CHCl_3$–MeOH or CH_2Cl_2–MeOH as eluting solvent. Spots on TLC were visualized under UV light and by spraying with anisaldehyde–H_2SO_4 reagent, followed by heating.

Plant material. Rhizomes of *C. comosa* were obtained from Saraburi Province and a voucher specimen (BKF No. 97298) is deposited at the Forest Herbarium, Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangkok.

Extraction and isolation. The sliced rhizomes were dried in an oven at 50–55°. The pulverized rhizome (1.3 kg) was extracted successively with *n*-hexane and EtOH in a Soxhlet apparatus. The concd EtOH extract was diluted with H_2O and extracted successively with EtOAc and *n*-BuOH. The EtOAc extract was chromatographed and four main frs were selected. The first fr was chromatographed twice to afford 15 mg of 1,7-diphenyl-5-hydroxy-(1*E*)-1-heptene (**3**) as an amorphous solid (lit. [4] mp 44–46°). ^{1}H NMR and EI-MS data were consistent with those reported for **3** [4, 7]. The second fr after CC (×3) yielded 24 mg of 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1*E*)-1-heptene (**6**). TLC comparison with an authentic sample, and the IR and ^{1}H NMR data were identical to those reported for compound **6** [5]. The third fr was similarly subjected to repeated CC to give 44 mg of 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1*E*)-1-heptene (**7**), mp 99–100°, from $CHCl_3$–C_6H_6 (lit. [5] mp 100–102°). TLC comparison with an authentic sample, and the IR and ^{1}H NMR data were identical to those reported for compound **7** [5].

The BuOH extract was subjected to CC (×3). TLC comparison of the fourth fr from the EtOAc extract with one of the selected frs from the BuOH extract suggested the identity of the major component of these two frs. Repeated CC of the combined fr resulted in the isolation of a new phloracetophenone glucoside, 4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl)acetophenone (**8**) (22 mg), mp 222–224°. Found: C, 50.99; H, 5.45. $C_{14}H_{18}O_9$ requires: C, 50.90; H, 5.45%. $[\alpha]_D^{20}$ −84.5 (MeOH, *c* 0.20). UV λ_{max}^{MeOH} nm (log ε): 220 (4.31), 285 (4.33). IR ν_{max}^{KBr} cm^{-1}: 3398, 2924, 1625 (*br*), 1514, 1461, 1367, 1282, 1199, 1173, 1072, 1038, 826. ^{1}H NMR ($CDCl_3$): δ 2.68 (*s*, 3H, CH_3); 3.37–3.55 (*m*,

4H), 3.71 (*dd*, J = 12.1, 4.9 Hz, 1H), and 3.90 (*dd*, J = 12.1, 1.8 Hz, 1H) (H-2′ to H-6′); 5.01 (*d*, J = 7.4 Hz, H-1′); 5.93 (*d*, J = 2.2 Hz, 1H, H-5); 6.17 (*d*, J = 2.2 Hz, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 33.4 (CH_3), 62.2 (C-6′), 71.0 (C-4′), 74.6 (C-2′), 78.3 and 78.4 (C-3′ and C-5′), 95.3 (C-5), 98.1 (C-1′), 101.9 (C-3), 106.7 (C-1), 162.6 (C-6), 166.2 (C-2), 167.7 (C-4), 204.7 (CO). EI-MS *m/z* (rel. int.): 330 $[M]^+$ (0.2), 312 $[M - H_2O]^+$ (0.4), 168 (96), 153 (100).

Bioassay. The effect of the phloracetophenone glucoside **8** on bile flow rate was evaluated in rats as described in [3]. Intraduodenal administration of **8** at doses of 25 and 50 mg/kg body weight stimulated bile flow rate to 125.3 ± 2.6 and 142.3 ± 3.0% of control, respectively. The onset of action was rapid and peaked at 30 min after administration.

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