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3′-FORMYL-2′,4′,6′-TRIHYDROXY-5′-METHYLDIHYDROCHALCONE, A PROSPECTIVE NEW AGROCHEMICAL FROM *PSIDIUM ACUTANGULUM*

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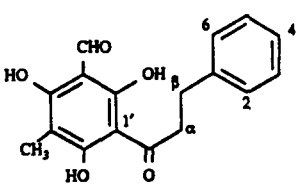
ABSTRACT.—CH_2Cl_2 extracts of the leaves of *Psidium acutangulum* revealed potent antifungal activity. A compound was isolated which demonstrated antifungal activity and tobacco budworm antifeedant activity. Structural elucidation studies indicated that the new compound is 3′-formyl-2′,4′,6′-trihydroxy-5′-methyldihydrochalcone [**1**].

A search for compounds from plants of the Amazon River basin of Peru was conducted in regard to activity as insect antifeedants or antifungal agents. One of the plants which demonstrated activity was *Psidium acutangulum* DC. (Myrtaceae). $CHCl_2$ extracts of the leaves of *Ps. acutangulum* revealed potent antifungal activity when tested against *Rhizoctonia solani, Helminthosporium teres,* and *Pythium ultimum* (1). No chemical study has been undertaken on this plant. However, the constituents of the fruits of *Psidium gualava* (2–6), *Psidium cattelianum*, and *Psidium lucidum* (7) have been examined. The heartwood and roots of *Ps. gualava* have also been studied (8,9).

In this paper, we report the isolation and structure determination of the new compound 3′-formyl-2′,4′,6′-trihydroxy-5′-methyldihydrochalcone [**1**] from *Ps. acutangulum*. Compound **1** showed antifungal activity against *R. solani* (132% zone of inhibition versus dithane standard with a threshold concentration of 2.1 $\mu g/cm^2$) and antibacterial activity against *Xanthomonas campestris* (114% zone of inhibition versus dithane standard with a threshold concentration 1.3×10^{-6} $\mu g/cm^2$). Significant antifeedant activity (90% feeding control at dose 0.25 mg/cm^2) was also demonstrated against the tobacco budworm (*Heliothis virescens*) by using the Hedin method (10).



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Compound **1** was obtained by repetitive crystallization from MeOH from a fraction obtained by rechromatography on Si gel of the EtOH extract of *Ps. acutangulum*. Compound **1** gave a molecular formula of $C_{17}H_{16}O_5$ by hrms, which indicated ten degrees of unsaturation. The presence of aromaticity in the molecule was suggested by fragmentations at *m/z* 77 (phenolic group) and 91 (benzylic group).

The uv spectrum was characteristic of flavonoids as indicated in Table 1 (11–14). The two triplets appearing in the ^{1}H-nmr spectrum were centered at δ 3.02 (2H, H-β) and at δ 3.46 (2H, H-α). This suggested the presence of a dihy-

TABLE 1. Color Reactions of Compound **1**.

Reagent	Visible light	Uv light
NH_3 vapor	pure yellow	pure yellow
5% ethanolic $AlCl_3$	orange	brown
5% aqueous Na_2CO_3	orange	—
1% $FeCl_3$/1% $K_3Fe(CN)_6$	blue	—

drochalcone since only the protons at C-β and C-α would give signals similar to those described above. The five-proton singlet at δ 7.28 could be assigned to the five protons of a benzene ring. However, a nonsubstituted A ring could be ruled out since the presence of the keto group attached at C-1′ would deshield strongly the protons of C-2′ and C-6′; thus they would appear in the ^{1}H nmr at δ 7.8–8.0 and the ones at C-3′, C-4′, and C-5′ at δ 7.1–7.7. Therefore, the five-proton singlet at δ 7.28 could be assigned to the five protons of ring B. The ms fragmentation pattern (*m/z* 91 and 104) was also in accord with an unsubstituted B ring. When compound **1** was methylated, the eims spectrum of the product gave a molecular ion $[M]^+$ at *m/z* 342, which corresponded to the methylation of three hydroxyl groups. The three-proton singlet which appeared at δ 2.05 could be assigned to a methyl group next to a double bond or aromatic ring. Thus, from the molecular formula $C_{17}H_{16}O_5$, it was evident that only -CHO had not been assigned. The carbon appearing at δ 192.18 in the ^{13}C nmr of compound **1** could be assigned to a carbonyl group (besides the one appearing at δ 186.74, which was characteristic of the dihydrochalcone carbonyl group). On the basis of the above chemical and spectral data, compound **1** is a dihydrochalcone in which the B ring is nonsubstituted and the A ring contains three hydroxyl groups, one carbonyl group, and a methyl group next to a double bond.

The presence of a methyl group as an aromatic substituent and not as a methyl ketone was confirmed by a negative iodoform test. On the other hand, the presence of an aromatic aldehyde was confirmed by a positive Tollens' test and by a negative reaction with Benedict's solution. The one-proton singlet appearing in the ^{1}H-nmr spectrum of compound **1** at δ 10.11 could, therefore, be assigned to the aldehyde proton.

The ir spectrum showed one absorption band for a carbonyl at 1600 cm^{-1}. The low value for this absorption frequency can be explained by the presence of hydroxyl groups ortho to the aromatic carbonyl groups. The low field shifts for two hydroxyl protons (δ 14.36 and 15.25) were in accord with strong hydrogen bonding with ortho carbonyl substituents (15). The bathochromic shift observed by the addition of $AlCl_3$ is in accord with a 6′-hydroxydihydrochalcone (10–14).

An X-ray crystallographic analysis was performed and was in accord with structure **1**. Therefore, compound **1** is 3′-formyl-2′,4′,6′-trihydroxy-5′-methyldihydrochalcone. Compounds such as **1**, which have demonstrated antifungal, antibacterial, and tobacco budworm antifeedant activity (Table 2), may offer potential as natural agrochemicals.

TABLE 2. Antifeedant Activity of Compound **1** Against *Heliothis virescens*.[a]

Time (days)	Concentration (μg/cm²)			
	247.5	76.7	24.8	7.7
2	+++	+++	+++	++
4	++	++	++	++
6	++	++	+	+

[a]+++ corresponds to 90–100% feeding control; ++ corresponds to 60–90% feeding control; + corresponds to 30–60% feeding control; % feeding control = $\frac{\%\text{ feeding sample}}{(1-\%\text{ feeding blank})} \times 100$.

EXPERIMENTAL

ISOLATION OF 3′-FORMYL-2′,4′,6′-TRIHYDROXY-5′-METHYLDIHYDROCHALCONE [**1**].—*Ps. acutangulum* was collected from Peru in 1982 and was identified by Manuel Rimachi of the Institute of Botanical Exploration, Mississippi State University. An herbarium specimen (voucher no. 3324) is filed in the Department of Biological Sciences at Mississippi State University. The dried, chopped twigs and leaves of *Ps. acutangulum* (28

kg) were extracted for 16 h in a Soxhlet extractor with CH_2Cl_2 followed by evaporation of the CH_2Cl_2 in vacuo. This fraction was chromatographed on Si gel (400 g). The column was eluted with $C_6H_6/CHCl_3$ solvent systems. The 50% $CHCl_3/C_6H_6$ fractions were combined and evaporated to yield 10.4 g. This portion showed the highest antifungal activity. This fraction was rechromatographed on Si gel (500 g) using $C_6H_6/CHCl_3$ as a solvent. The 75% $CHCl_3/C_6H_6$ extract afforded an antifungal active fraction (7.2 g) which was rechromatographed once more under the same conditions on Si gel (180 g). The 100% C_6H_6 fraction was purified by repetitive crystallization from MeOH to yield 800 mg of white needles: mp 157–158°; $[\alpha]^{25}D$ 0° ($c = 0.2$, $CHCl_3$); ir (KBr) 3310, 3060, 3000, 2890, 2650, 1600, 1430, 1380, 1180 cm^{-1}; uv (MeOH) 288 (log ε 4.45) and 384 (log ε 3.89), (NaOMe) 293 (log ε 4.46) and 382 (log ε 3.58), ($AlCl_3$) 298 (log ε 4.54) and 370 (log ε 3.75), ($AlCl_3$ and HCl) 294 (log ε 4.56) and 364 (log ε 3.78), (NaOAc) 290 (log ε 4.45) and 384 (log ε 3.92), (NaOAc and H_3BO_3) 295 (log ε 4.38) and 396 (log ε 3.94). *Anal.* calcd for $C_{17}H_{16}O_5$: C 67.99, H 5.37; found C 67.80, H 5.49. Mol wt hrms *m/z* 300.10081; calcd for $C_{17}H_{16}O_5$, 300.09978.

Other significant peaks: hrms *m/z* (rel. int. %) 300 (100), 282 (76), 253 (28), 209 (50), 195 (80), 168 (75), 104 (57), 91 (72), 77 (55); ^1H nmr (200 MHz, $CDCl_3$) δ 2.05 (3H, s, H-5′), 3.02 (2H, t, $J = 7.5$ Hz, H-β), 3.46 (2H, t, $J = 7.5$ Hz, H-α), 7.28 (5H, m, Ar), 10.11 (1H, s, H-3′), 14.36 (1H, s, OH), 15.25 (1H, s, OH); ^{13}C nmr (200 MHz, $CDCl_3$) 15.4 (C-5′), 30.3 (C-α), 45.5 (C-β), 9.38 (C-3′), 103.7 (C-5′), 125.9 (C-4), 128.3 (C-2, C-3, C-5, C-6), 130.0 (C-2′, C-4′, C-6′), 141.2 (C-1), 186.8 (CHO), 192.92 (C=O).

METHYLATION OF COMPOUND **1**.—Compound **1** (4 mg) dissolved in MeOH (3 ml) was treated with an excess of CH_2N_2 in Et_2O (17) to yield the methylated compound: mp 140–141°; ir (KBr) 3060, 3020, 1725, 1620, 1450, 1070 cm^{-1}; uv (MeOH) λ max 285 nm (log ε 4.37), 340 (log ε 3.71); eims *m/z* (%) $[M]^+$ 342 (30), 311 (23), 251 (45), 237 (100), 210 (27), 149 (36), 105 (27), 91 (59).

X-RAY DATA FOR **1**.—A fragment of a needle-shaped crystal of compound **1**, with dimensions 0.4 × 0.1 × 0.05 mm, was used for all measurements. The data were collected with an Enraf-Nonius CAD-4 diffractometer using graphite monochromated. The crystals were orthorhombic, space group $P2_12_12_1$. The unit cell parameters (16) were: $a = 12.077(2)$ Å, $b = 21.340(7)$ Å, $c = 5.615(2)$ Å, U = 1447 Å^3, Z = 4, $D_x = 1.383$ g/cm^3, linear absorption coefficient $\mu = 0.84$ cm^{-1}. Data were collected by ω–2θ scans of variable scan speed. The orientation was checked every 100 reflections. Three standard reflections were monitored every 2.5 h of exposure. No decay was observed. We collected 1224 unique reflections, of which 380 had $I > 3\sigma$ (I) and were used in the refinement. The structure was solved using the program MULTAN (17) and refined using isotropic thermal parameters for non-hydrogen atoms. Unit weights were used in all stages of refinement and final R = 0.060 and Rw = 0.058 were obtained. The positions of the hydrogen atoms bonded to carbon atoms were calculated except for those on the methyl groups, which were located in a difference Fourier map and idealized based on known geometry. The final difference Fourier synthesis showed no significant features [0.25 e·Å^{-3}]. The positions of the H atoms of the hydroxyl groups were also found in the Fourier map but they have to be considered tentative. The final calculations were performed using SHELX.[1]

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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