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A NEW BIOLOGICALLY ACTIVE PHENOLIC FROM *CATTLEYA TRIANAEI**

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Key Word Index—*Cattleya trianaei*; Orchidaceae; phenolic; eucomic acid; hydroxyeucomic acid; tyramine; dopamine; biological activity.

Abstract—A new phenolic, hydroxyeucomic acid, and dopamine were isolated from *Cattleya trianaei* and their biological activities examined.

INTRODUCTION

Recently, it has been demonstrated that the multiplication of orchids can be carried out by shoot tip culture [1]. In the case of *Cattleya*, however, the medium solution occasionally changes to reddish brown and the growth of the shoot tip is gradually inhibited during the shoot tip culture. Eucomic acid was previously isolated from the growth inhibitory fraction of *Cattleya trianaei* 'Mooreana' in addition to tyramine which had no biological effects. Eucomic acid was effective at a concentration of 5 ppm for the assay of growth inhibition using seedlings of *Sophrolaeliocattleya* (Slc) Jane Miyoshi (Gertie) [2]. Eucomic acid has also been isolated from *Petalostemon gattingeri* [3], *Eucomis punctata* [4] and *Lycoris radiata* [5] and assayed for inhibition of germination [3] and auxin-like activity [5].

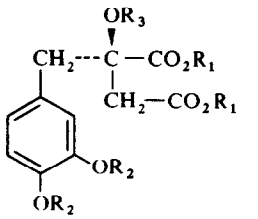
This paper deals with the isolation of a new phenolic from *Cattleya trianaei* and its biological assay.

RESULTS AND DISCUSSION

When the H_2O extract of fresh *Cattleya* shoots was fractionated as previously described [2], the *n*-BuOH extractives showed a small amount of a new phenolic giving a positive reaction with diazotized benzidine (orange) and $FeCl_3$ reagent (greenish brown), in addition to eucomic acid. However, when fresh plant material was homogenized and incubated at 30° for 17 hr in phosphate buffer, substantial amounts of the new phenolic were generated instead of eucomic acid.

After incubation and *n*-BuOH–H_2O partition, the *n*-BuOH extractives were subjected to repeated column chromatography on Sephadex LH-20, Silica gel 60 and Avicel to give a new compound, **1**, colourless needles, $C_{11}H_{12}O_7$, mp 159–162°, $[\alpha]_D$ −14° (in MeOH). **1** gave an orange colour with diazotized benzidine, a red colour with the Griess test and greenish-brown colour with $FeCl_3$ reagent. The UV spectrum indicated the presence of a non-conjugated benzene ring (283 nm), and the IR spectrum a OH (3300 cm^{-1}), CO (1740 cm^{-1}) and a benzene ring (1610 cm^{-1}). The ^1H NMR spectrum exhibited the presence of 3 phenyl protons (δ 6.68–6.88, 3H, *m*) and of two pairs of methylene groups (δ 2.68, 2.94, 2H, each *d*, J = 20 Hz and 2.72, 2.96, 2H, each *d*, J = 16 Hz).

1 when treated with CH_2N_2 in MeOH gave a diMe ester, **2**, as a colourless syrup, $C_{13}H_{16}O_7$, $[\alpha]_D$ −18° (in $CHCl_3$), which had two new carbomethoxyl signals (δ 3.65, 3.73, 3H × 2) and ABX type signals (δ 6.49, 1H, *dd*, J = 2 and 8 Hz; 6.72, 1H, *dd*, J = 1 and 8 Hz; 6.72, 1H, *dd*, J = 1 and 2 Hz) attributable to phenyl protons, in addition to two methylene signals. **2** has an alcoholic OH group as inferred from its MS which shows the loss of H_2O from the M^+ and its fragmentation pattern is similar to that of eucomic acid diMe ester but with the increase of 16 amu [2]. This evidence strongly suggested that **1** might be hydroxylated eucomic acid.



1 $R_1 = R_2 = R_3 = H$
2 $R_1 = Me$, $R_2 = R_3 = H$
3 $R_1 = Me$, $R_2 = R_3 = Ac$

Acetylation of **2** using Ac_2O and *p*-TsOH gave a triacetate, **3**, as colourless syrup, which showed no OH absorption in the IR but two kinds of acetoxyl ^1H NMR signals, one alcoholic (δ 2.12, 3H, *s*) and the other phenolic (δ 2.31, 6H, *s*). The MS of **3** gives a M^+ at *m/e* 410 and ions at *m/e* 378, 349, 308, 266, 234, 206 and 123 in full agreement with its structure as eucomic acid diMe ester diacetate [2].

* Part 2 in the series "*Cattleya*". For Part 1 see ref. [2]. Part of this work was presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan.

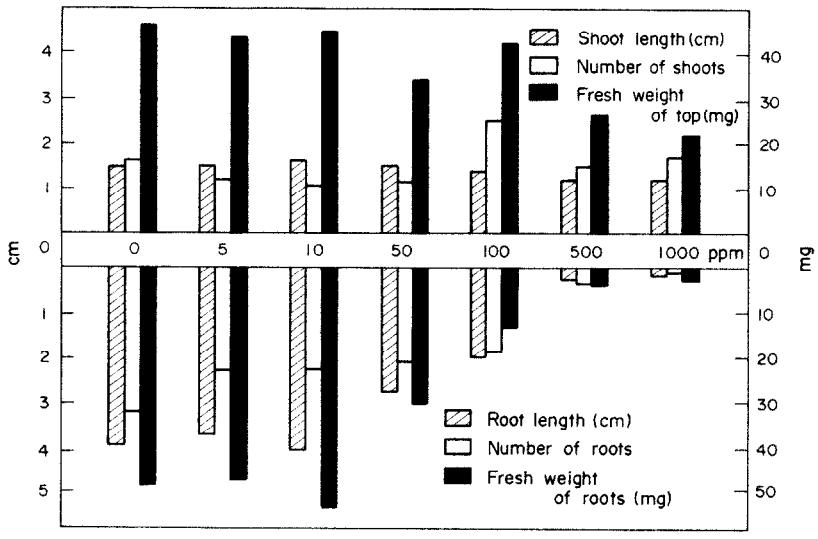


Fig. 1. Effect of hydroxyeucomic acid on growth of *Cattleya* seedlings.

From all the above facts, the structure of **1** has been confirmed as the hydroxyeucomic acid and we so name it.

Another compound, **4**, which was isolated from *Cattleya* using an alkaline media, appeared to be related to tyramine. It gave a pink-violet colour with ninhydrin, brownish-orange with diazotized benzidine, greenish-gray with $FeCl_3$ and had similar TLC R_f values on Si gel and cellulose. After acetylation of the alkaline fraction, **5** was isolated by PLC and then identified by direct comparison with an authentic dopamine triacetate (UV, IR and MS). This is the first identification of dopamine from *Cattleya* species.

Bioassay

The results after 14 weeks cultivation of *Cattleya* seedlings in a medium containing **1** are shown in Fig. 1. As we reported previously [2], eucomic acid has an inhibitory effect on the growth of shoot and root of the seedlings at a concentration of 5 ppm and a parallel growth inhibition occurs on both shoot and root. However, in the case of **1**, although strong inhibition was observed on the elongation, fr. wt and number of roots at a concentration of 500 ppm, the growth inhibition of the shoot was not effective between 5 ppm and 100 ppm. Since it is well known that *o*-diphenols are inhibitory for IAA oxidase activity [6], this is to be expected. Addition of rutin (50 ppm) abolishes the inhibitory effect of **1**. Finally, we tested **1** in the oat coleoptile test and found it was inactive.

EXPERIMENTAL

All mps are uncorr. ^{1}H NMR spectra were measured at 100 MHz and chemical shifts are given on δ (ppm) scale with TMS as the internal standard. PC and TLC were carried out using *n*-BuOH–HOAc–H_2O (4:1:5, upper layer) as a solvent. Diazotized benzidine reagent, $FeCl_3$ reagent, I_2 vapour, ninhydrin reagent, 10% H_2SO_4, Griess reagent and UV were used for detection. Column chromatography was carried out with Sephadex LH-20 (25–100 μm), Si gel 60 (60–200 μm, Merck) and Avicel using MeOH for the first and *n*-BuOH–HOAc–H_2O (4:1:5, upper layer) for the others.

Isolation of **1**. Fresh leaves and stems (80 g) of *Cattleya trianaei* were homogenized with 0.1 M K_2HPO_4 (800 ml) and 0.1 M $MgCl_2$ (10 ml) added. The mixture (pH 7.5) was incubated for 17 hr at 30°. The incubated soln was filtered. The filtrate was adjusted to pH 2 with HCl and then extracted with $CHCl_3$. The aq. layer was then repeatedly extracted with *n*-BuOH. The *n*-BuOH extractive (812 mg) was subjected to Si gel, Sephadex and finally Avicel column chromatography to give a pale yellow syrup (46.7 mg) which gave colourless crystalline **1** after recrystallization from $CHCl_3$–MeOH. **1** gave an orange colour with diazotized benzidine reagent and a greenish-brown one with $FeCl_3$, mp 159–162°, $[\alpha]_D^{20}$ −14° (*c* 1.16, MeOH). (Found: C, 51.73; H, 4.81. $C_{11}H_{12}O_7$ requires: C, 51.56; H, 4.72%). UV λ_{max}^{MeOH} nm (log ε): 283 (3.51); IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1740 (C=O), 1610 (C=C). ^{1}H NMR (D_2O): δ 2.68, 2.94 (2H, *br d*, J_{gem} = 20 Hz), 2.72, 2.96 (2H, each *d*, J_{gem} = 16 Hz), 6.68–6.88 (3H, *m*, aromatic H).

Dimethyl ester of **1**. **1** was methylated with CH_2N_2 in MeOH. The product was passed through a Si gel column using hexane–EtOAc (1:2) as a solvent to give a colourless syrup, **2**. $[\alpha]_D^{23}$ −18° (*c* 2.35, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 283 (3.56). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3400 (OH), 1740 (C=O), 1615 (C=C). ^{1}H NMR ($CDCl_3$): δ 2.73, 3.04 (2H, each *d*, J_{gem} = 20 Hz), 2.81, 2.94 (2H, each *d*, J_{gem} = 17 Hz), 3.65, 3.73 (3H × 2, each *s*, —CO_2Me), 6.49 (1H, *dd*, *J* = 2 and 8 Hz), 6.72 (1H, *dd*, *J* = 1 and 8 Hz), 6.72 (1H, *dd*, *J* = 1 and 2 Hz). (Found: M$^+$ 284.0915. $C_{13}H_{16}O_7$ requires 284.0896). MS *m/e*: 284 (M$^+$), 266 (M$^+$ − H_2O), 253 (M$^+$ − OMe), 234 (M$^+$ − H_2O − OMe), 225 (M$^+$ − CO_2Me), 206 (M$^+$ − H_2O − CO_2Me), 193 (M$^+$ − CO_2Me − OMe).

Acetylation of **2**. **2** (27.5 mg) was acetylated with Ac_2O (1 ml) containing *p*-TsOH (10 mg) by heating at 95° for 2 hr. The reaction mixture was washed × 5 using Folch's method [7] and then evapd *in vacuo* to give a colourless syrup, **3** (31.2 mg). UV λ_{max}^{MeOH} nm (log ε): 207 (4.46), 266 (3.31 sh), 272 (3.42), 281 (3.24 sh). IR ν_{max}^{film} cm^{-1}: 1770, 1745 (C=O), 1610, 1595 (C=C). ^{1}H NMR ($CDCl_3$): δ 2.12 (3H, *s*, OAc), 2.31 (6H, *s*, 2OAc), 2.92, 3.17 (2H, each *d*, J_{gem} = 20 Hz), 3.28, 3.45 (2H, each *d*, J_{gem} = 17 Hz), 3.68, 3.72 (3H × 2, each *s*, CO_2Me), 6.84–7.16 (3H, *m*, aromatic H). MS *m/e*: 410 (M$^+$), 378, 349, 308, 266, 234, 206 and 123.

Isolation of dopamine triacetate. The H_2O layer was concd to 30 ml and adjusted pH 10 with conc NH_4OH. Free amines were extracted with EtOAc and then *n*-BuOH. Both layers were combined and the solvent evapd to give a brownish syrup (122 mg). This was dissolved in Ac_2O–Py (1:1) (4 ml) and allowed to stand overnight at room temp. The solvent was evapd *in vacuo* and the residue extracted with EtOAc. The EtOAc extractives (180 mg) were purified by PLC on Si gel 60 (detection by UV) using $CHCl_3$–MeOH (10:1) as solvent and the bands removed and extracted with $CHCl_3$–MeOH (1:1) to give dopamine triacetate (1.4 mg) and tyramine diacetate (35 mg). Dopamine triacetate was identified by the comparison with an authentic sample (UV, IR and MS).

Biological assay. Reinert and Mohr medium [8] containing **1**, dopamine and the mixture of eucomic acid and rutin (50 mg) was prepared as previously described [2]. Ten young *Cattleya* seedlings which had two leaves and were 8 mm high were cultivated for 14 weeks at 25° under 2000 lx as previously described. The results are given in the text. The Avena test was carried out with oat coleoptiles (5 mm sections cut 3 mm below the tip), 20 sections per dish floated on 1 ml of the test solutions.

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