RESEARCH ARTICLE

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CHEMICAL CONSTITUENTS OF *DIANELLA ENSIFOLIA* REDOUTE

VITCHU LOJANAPIWATNA, KOVIT CHANCHAROEN, KANCHANA SAKARIN and PICHAET WIRIYACHITRA

Department of Chemistry, Prince of Songkla University, Hat Yai, Thailand.

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Abstract

Musizin (dianellidin) (1), methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate (8), methyl 2,4-dihydroxy-3,6-dimethylbenzoate (7), methyl 2,4-dihydroxy-6-methylbenzoate (methyl orsellinate)(9),2,4-dihydroxy-6-methoxy-3-methylacetophenone (14),5,7-dihydroxy-2,6,8-trimethylchromone (11) and 5,7-dihydroxy-2,8-dimethylchromone (isoeugenitol) (13) have been isolated from the roots of Dianella ensifolia *Redoute.*

Introduction

The genus *Dianella,* which belongs to the family Liliaceae, comprises 30 species which are distributed throughout tropical Asia, Australia, Polynesia and New Zealand[1], *Dianella ensifolia* Redoute, a grass-like plant which occurs widely in the vicinity of the Prince of Songkla University, is the only species that has been recorded in Thailand[2]. This plant is apparently not used in traditional Thai medicine. However, according to the local people in Surat Thani province, Southern Thailand, its roots have been used for the treatment of kidney diseases.

A survey of literature[3-9] revealed that chemical work has been carried out on only a few *Dianella* species and most of these have been studied by R.G. Cooke and his coworkers in Australia.

These workers found that the roots of *D. laevis* contained musizin or dianellidin (1)[3] and its glycoside, dianellin (2)[3]. The quinones dianellinone (3)[4] and trianellinone (4)[5,6] were later isolated from *D. revoluta.* Further examination of the roots of this species resulted in the isolation of an optically active flavan derivative (6)[6,7], the substituted benzoate (7)[6] and a mixture of long-chain substituted chromone homologues (10)[6,8].

Dr. R.G. Cooke (private communication) has isolated another flavan (5), an acetophenone (14) and chrysophanol (15) from *D. tasmanica.*

In addition to the above studies, Briggs, Briggs and King found that dianellidin (1) and dianellin (2) were also present in *D. nigra*[9].

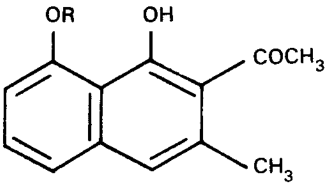
So far there has been no chemical investigation on *D. ensifolia.* We encountered this species during a survey of Thai plants for the presence of alkaloids[10]. Although this plant gave a negative test, it was noticed that the roots were yellow. It was then decided to investigate these pigments.

In the present work, the roots of *D. ensifolia* were extracted with benzene and chloroform to give two main fractions.

The benzene extract was fractionated with aqueous $NaHCO_3$ and aqueous NaOH. Chromatography of the sodium bicarbonate soluble fraction led to the isolation of musizin (1) while the sodium hydroxide soluble fraction yielded methyl 2,4-dihydroxy-3,5,6-trimethybenzoate (8), methyl orsellinate (9), 2,4-dihydroxy-6-methoxy-3-methylacetophenone (14), 5,7-dihydroxy-2,6,8-trimethylchromone (11) and 5,7-dihydroxy-2,8-dimethylchromone (13).

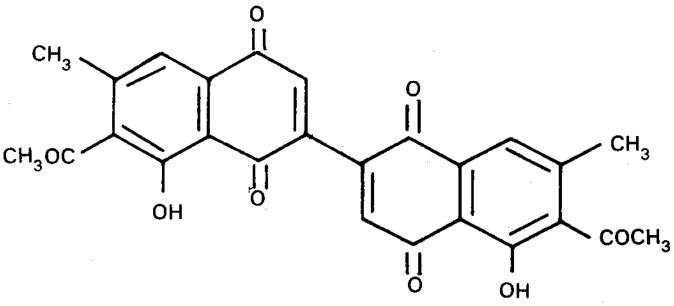
Extensive chromatography of the chloroform extract gave methyl 2,4-dihydroxy-3,6-dimethylbenzoate (7).

It was noticed that the compounds obtained previously fell broadly into three different groups : derivatives of naphthalene, benzoic acid and acetophenone; the latter also included the chromones; no quinones or flavans were found to be present in the roots of this species.



(1) R = H

(2) R = rhamnose-glucose-



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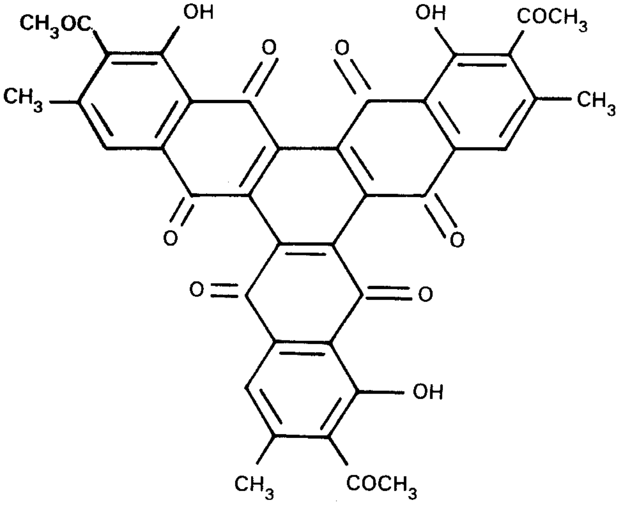

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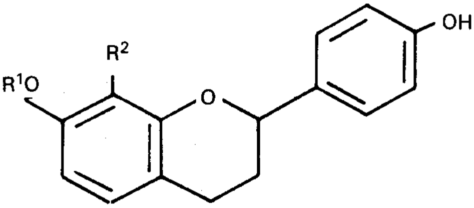
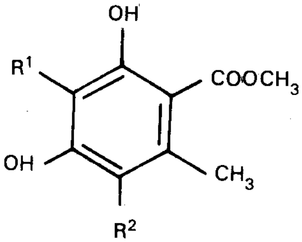
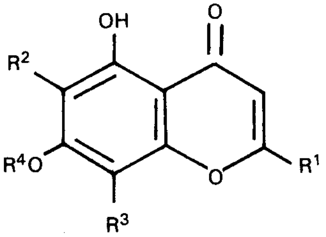


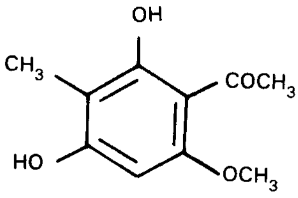
(5) $R^1 = R^2 = H$
(6) $R^1 = R^2 = CH_3$



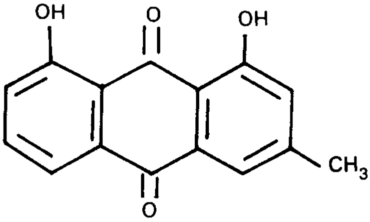
(7) $R^1 = CH_3, R^2 = H$
(8) $R^1 = R^2 = CH_3$
(9) $R^1 = R^2 = H$



(10) $R^1 = C_{27}H_{55}, C_{29}H_{59}$ and $C_{31}H_{63}$, $R^2 = CH_3; R^3 = R^4 = H$
(11) $R^1 = R^2 = R^3 = CH_3; R^4 = H$
(12) $R^1 = R^2 = R^3 = R^4 = CH_3$
(13) $R^1 = R^3 = CH_3; R^2 = R^4 = H$



(14)



(15)

Musizin (1) which is a naphthalene derivative was identified by its physical properties and by direct comparison with an authentic specimen.

The substituted benzoates (7), (8) and (9) also proved to be identical with authentic specimens.

Although 2,4-dihydroxy-3,5,6-trimethylbenzoic acid has been isolated from the fungus *Mortierella ramanniana* var. *angulispora* (Naumov) Linnemann[11], the corresponding methyl ester (8)[12] has apparently not been recognized as a natural product previously.

The structures of the acetophenone (14) and the chromone (11) were also established by comparison with authentic specimens; the chromone (13) was identified on the basis of its physical properties.

Compound (11) which is the O-demethyl derivative of angustifolionol (12)[13,14] was isolated from *Marsdenia tinctoria* var. *tomentosa* Masamune[15]. The chromone (13), isoeugenitol, has been isolated previously from *Eugenia caryophyllata* (L.) Thunbg. V[16].

Experimental

Melting points were determined on an Buchi Tottoli melting point apparatus. Ultraviolet spectra were recorded with a Beckman DK-2A instrument. Mass spectra were measured with a Hewlett Packard 5986 spectrometer and nuclear magnetic resonance (n.m.r.) spectra were obtained with a Bruker WP80 spectrometer operating at 80 MHz for Fourier transform ^{1}H spectra.

A voucher specimen of the plant material (JRC 313) has been deposited in the Herbarium of the Biology Department, Prince of Songkla University, Hat Yai, Thailand.

Dried roots (3kg) of plants which had been collected in the vicinity of the Prince of Songkla University were extracted continuously (Soxhlet) with benzene (10 l) and then with chloroform (10 l) for 3 days (at room temperature.) Each extract was concentrated under reduced pressure to a brown tar.

The fraction (34.9 g) which was extracted with benzene was dissolved in ether (3 l) and the soution was shaken successively with 5% aq. $NaHCO_3$ (3 × 500 ml) and 5% aq. NaOH (3 × 500 ml). After drying over anhydrous Na_2SO_4, the ether layer was evaporated to yield a waxy residue (5.1 g).

a) Isolation of musizin (1)

The 5% $NaHCO_3$ extract was acidified and then extracted with ether to give a dark brown solid (12.2 g) which was chromatographed over silicic acid (350 g) using benzene, benzene/chloroform, chloroform and chloroform/methanol as eluting agents. Musizin (1) was readily eluted with benzene/chloroform (1:1) and it crystallized from acetone/'light petroleum as bright yellow needles (150 mg), m.p. 162–163.5°C (lit[3] 164°C), not depressed on admixture with an authentic sample supplied by Professor R.H. Thomson. High resolution mass spectrum: found M^+ 216.0783, calculated for $C_{13}H_{12}O_3$ M^+ 216.0783. λ_{max} (EtOH) : 224 (log ε 4.52), 263 (4.08), 291 (3.08), 303 (3.28), 317 (3.38), 337 (3.68), 350 (3.72) nm. N.m.r. ($CDCl_3$) δ : 1.61 (s, 1H, OH), 2.62 (s, 3H, $COCH_3$), 2.69 (s, 3H, $ArCH_3$), 6.91 (dd, 1H, ArH), 7.06 (s, 1H, ArH), 7.12 (dd, 1H, ArH), 7.42 (t, 1H, ArH), 10.35 (s, 1H, OH). Mass spectrum m/e : 216 (71%), 201 (100), 198 (35), 155 (24), 127 (35), 115 (29).

b) Isolation of methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate (8), methyl orsellinate (9), 2,4-dihydroxy-6-methoxy-3-methylacetophenone (14), 5,7-dihydroxy-2,6,8-trimethylchromone (11) and 5,7-dihydroxy-2,8-dimethylchromone (13).

The 5% NaOH fraction was acidified and extracted with ether. Evaporation of the solvent then gave a dark-brown tar (14.4 g). This product was chromatographed over silicic acid (400 g) with light petroleum, light petroleum/benzene, benzene, benzene/chloroform, chloroform, chloroform/methanol. Fractions (150 ml) of the elute were collected and those with identical behaviour on t.l.c. were combined and evaporated. In this way 9 fractions were obtained.

The first fraction, consisting of a pale yellow waxy solid (199 mg), was not examined further.

The second fraction, eluted with light petroleum/benzene (9:1), yielded a pale yellow solid (188 mg) which travelled as a single spot on t.l.c. This substance was recrystallized from acetone/light petroleum to afford methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate (8) as yellow needles (63.9 mg), m.p. 95-96.5°C (lit[12] 96-97°C), not depressed on admixture with a synthetic specimen supplied by Associate Professor M.V. Sargent. High resolution mass spectrum : found M^+ 210.0892, calculated for $C_{11}H_{14}O_4$ 210.0891 λ_{max} (EtOH) : 217 (log ε 4.22), 271 (4.11), 313 (3.58) nm. N.m.r. ($CDCl_3$) δ : 2.20 (s, 6H, 2 × $ArCH_3$), 2.45 (s, 3H, $ArCH_3$), 3.95 (s, 3H, OCH_3), 5.16 (s, 1H, ArOH exchanged with D_2O), 11.47 (s, 1H, ArOH exchanged with D_2O). Mass spectrum m/e : 210 (28%), 178 (48), 150 (100).

Methyl 2,4-diacetoxy-3,5,6-trimethylbenzoate

The benzoate (8) (13.2 mg) was dissolved in pyridine (0.3 ml) and acetic anhydride (0.3 ml) then the reaction mixture was stirred at room temperature overnight. The mixture was worked up as usual and the product was recrystallized from methanol to afford methyl 2,4-diacetoxy-3,5,6-trimethylbenzoate as colourless plates (9.9 mg), m.p. 123-124°C (lit[11] 123-124°C). N.m.r. ($CDDl_3$) : 1.94 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.25 (s, 6H, 2 × $ArCH_3$), 2.35 (s, 3H, $ArCH_3$), 3.89 (s, 3H, OCH_3). Mass spectrum m/e : 294 (absent), 252 (10%), 210 (22), 178 (100), 150 (33).

The third, fourth and fifth fractions eluted from the column by light petroleum/

benzene did not yield crystalline products and they were not examined further.

The sixth fraction eluted from column with benzene afforded a pale yellow solid (481 mg) which showed two main spots with closely similar R_f values on t.l.c. The mixture was separated repeatedly by preparative layer chromatography using chloroform as developing solvent.

The product with high R_f value crystallized from acetone/benzene to give methyl 2,4–dihydroxy–6–methylbenzoate (9) as colourless needles (8 mg), m.p. 129-132°C (lit[17] 138-140°C), undepressed on admixture with a synthetic specimen. N.m.r. (d_6–acetone) δ : 2.46 (s, 3H, $ArCH_3$), 3.92 (s, 3H, OCH_3), 6.25 (AB_q, 2H, 2 × ArH), 11.6 (s, 1H, ArOH exchanged with D_2O). Mass spectrum m/e : 182 (32%), 150 (100), 122 (53), 94 (19).

Recrystallization of the substance with lower R_f value yielded 2,4-dihydroxy-6–methoxy-3-methylacetophenone (14) as pale yellow needles (13.5 mg), m.p.227-228°C (lit[18] 225°C). The i.r. spectrum of this compound is identical with that provided by Dr. R.G. Cooke. High resolution mass spectrum : found M^+ 196.0729, calculated for $C_{10}H_{12}O_4$ 196.0735. N.m.r. (d_6–DMSO) δ : 1.88 (s, 3H, $COCH_3$), 2.53 (s, 3H, $ArCH_3$), 3.80 (s, 3H, OCH_3), 6.06 (s, 1H, ArH), 14.19 (s, 1H, ArOH exchanged with D_2O). Mass spectrum m/e : 196 (32%), 181 (100), 166 (14), 149 (20).

The seventh fraction eluted from column yielded a dark yellow solid (315 mg). T.l.c. revealed that this consisted of a mixture of the sixth and eighth fractions. This crude material was not examined further.

The eighth fraction which was eluted from the column with benzene yielded a pale red precipitate on concentration. This product was collected by filtration (305 mg) and crystallized from acetone to afford 5,7-dihydroxy-2,6,8-trimethylchromone (11) as pale brown needles (75 mg), m.p. 287-288°C(lit[19] 284-286°C),not depressed on admixture with an authentic specimen supplied by Professor Ito Kazuo[15]. High resolution mass spectrum : found M^+ 220.0736, calculated for $C_{12}H_{12}O_4$ 220.0735. λ_{max} (EtOH) : 228 (log ε 4.03), 256 (4.11), 262 (4.13), 303 (3.70) nm. N.m.r. (d_6-DMSO) δ : 2.04 (s, 3H, $C = C\text{-}CH_3$), 2.16 (s, 3H, $ArCH_3$), 2.38 (s, 3H, $ArCH_3$), 6.13 (s, 1H, CH = C), 13.01 (s, 1H, ArOH exchanged with D_2O). Mass spectrum m/e : 220 (100%), 219 (68), 191 (19), 177 (8), 151 (7).

The ninth fraction which was eluted with benzene/chloroform (4:3) afforded a colourless semisolid (931 mg). The product was repeatedly crystallized from chloroform to yield 5,7-dihydroxy-2,8-dimethylchromone (13) as colourless needles (75 mg), m.p. 232-232.5°C(lit[20] 235-236°C).High resolution mass spectrum : found M^+ 206.0578, calculated for $C_{11}H_{10}O_4$ 206.0578. λ_{max} (EtOH) : 226 (log ε 3.93), 254 (4.09), 259 (4.10), 299 (3.54), 320 (3.38) nm. N.m.r. (d_6-DMSO) δ : 2.12 (s, 3H, $C = C\text{-}CH_3$), 2.40 (s, 3H, $ArCH_3$), 6.18 (s, 1H, CH = C), 6.29 (s, 1H, ArH), 12.78 (s, 1H, ArOH exchanged with D_2O). Mass spectrum m/e : 206 (100%), 205 (80), 177 (25), 165 (21), 163 (13), 138 (15), 137 (19).

Further elution of the column with $CHCl_3$, $CHCl_3$/MeOH, MeOH failed to give any crystalline product.

c) Isolation of methyl 2,4-dihydroxy-3,6-dimethylbenzoate (7)

The fraction which was extracted with $CHCl_3$ (23 g) was chromatographed over silicic acid (700 g) with light petroleum, light petroleum/benzene, benzene, benzene/chloroform, chloroform and chloroform/methanol as eluents. Each fraction (200 ml) was monitored by t.l.c. and those fractions with the same behaviour were combined.

Only the fourth fraction yielded a pure product. This fraction, which was eluted with light petroleum/benzene (1:1), afforded a pale yellow solid (169 mg) on evaporation. This product was dissolved in ether and extracted with 5% aq. $NaHCO_3$ and then 5% aq. NaOH.

The aq. $NaHCO_3$ extract, after acidification and extraction with ether, yielded a trace (4 mg) of waxy material.

The aq. NaOH fraction, worked up as usual, offorded an orange solid (160 mg) which was crystallized from acetone/light petroleum to give methyl 2,4-dihydroxy-3,6-dimethylbenzoate (7) as pale yellow needles (74 mg), m.p.141-143°C(lit[6] 143-144°C),not depressed on admixture with an authentic specimen supplied by Dr. R.G. Cooke. λ_{max} (EtOH) : 219 (log ε 4.12), 270 (4.19), 303 (3,21) nm. N.m.r. ($CDCl_3$) δ : 2.12 (s, 3H, $ArCH_3$), 2.48 (s, 3H, $ArCH_3$), 3.96 (s, 3H, OCH_3), 4.69 (s, 1H, ArOH exchanged with D_2O), 6.24 (s, 1H, ArH), 12.03 (s, 1H, ArOH exchanged with D_2O). Mass spectrum m/e : 196 (37%), 165 (19), 164 (83), 136 (100), 135 (11).

Acknowledgement

We are grateful to Miss Puangpen Sirirugsa for identitying the plant material, to Dr. R.G. Cooke for authentic samples of compounds (7) and (14), to Professor R.H. Thomson for a sample of musizin (1), to Professor Ito Kazuo for an authentic sample of the chromone (11) and to Associate Professor M.V. Sargent for a generous gift of (8) and methyl dibromoorsellinate used to prepare methyl orsellinate (9). We are also grateful to Dr. Lindsay T. Byrne and Dr. W.C. Taylor for spectroscopic data. We thank the National Research Council of Thailand and the International Foundation for Science for financial support.

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บทคัดย่อ

จากการศึกษาส่วนประกอบทางเคมีของรากต้นยาหนูต้น (*Dianella ensifolia* Redoute) พบว่ามี **musizin** (dianellidin)(1), methyl 2,4-dihydroxy-3-5,6-trimethylbenzoate (8), methyl 2,4-dihydroxy-3,6-dimethylbenzoate (7), methyl 2,4-dihydroxy-6-methylbenzoate (methy orsellinate)(9),2,4-dihydroxy-6-methoxy-3-methylacetophenone (14),5,7-dihydroxy-2,6,8-trimethylchromone (11) และ 5,7-dihydroxy-2,8-dimethylchromone (isoeugenitol) (13).