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TWO FUNGICIDAL PHENYLETHANONES FROM *EUODIA LUNU-ANKENDA* ROOT BARK

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Key Word Index—*Euodia lunu-ankenda*; Rutaceae; root bark, fungicidal activity; furoquinoline alkaloids, phenylethanones, lupeol, bergapten

Abstract—*Euodia lunu-ankenda* root bark contained two fungicidal phenylethanones, 1-[2′,4′-dihydroxy-6′-(3″-methyl-2″-butenyloxy)-5′-(3″-methyl-2″-butenyl)]phenylethanone and 1-[2′,4′-dihydroxy-6′-(3″,7″-dimethylocta-2″,6″-dienyloxy)-5′-(3″-methyl-2″-butenyl)]phenylethanone, a known phenylethanone, five furoquinoline alkaloids, lupeol and bergapten.

INTRODUCTION

Euodia lunu-ankenda, a species found in southern Asia is used in the indigenous medicine of Sri Lanka [1]. Previous work includes the isolation of a chroman, three chromenes, a quinolinone and evolitrine from its aerial parts [2] and two more furoquinoline alkaloids, dictamine and kokusaginine and the furocoumarin, marmesin from its stem bark [3]. We now report the isolation of three phenylethanones, two of which are new, five furoquinoline alkaloids, lupeol and bergapten from its root bark.

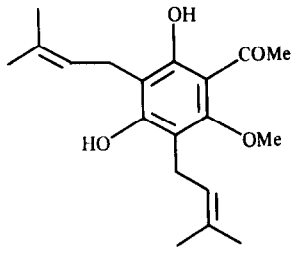
RESULTS AND DISCUSSION

The basic fraction of the dichloromethane extract of the root bark of *Euodia lunu-ankenda* gave on chromatographic separation the five furoquinoline alkaloids, dictamine, evolitrine, γ-fagarine, skimmianine and kokusaginine. The neutral fraction was shown to be strongly active against the fungus *Cladosporium cladosporioides* using the TLC bioassay technique [4]. Chromatography of the extract gave lupeol, 1-[2′,4′-dihydroxy-3′,5′-di(3″-methyl-2″-butenyl)-6′-methoxy)]phenylethanone (**1**) which has also been isolated from *Achronychia pedunculata* [5], two new phenylethanones (**2** and **3**) and the coumarin, bergapten. The fungicidal activity was found to reside in compounds **2** and **3**.

The UV λ_{max} of phenylethanones **2** and **3** supported the presence of a phloroglucinol type of chromophore [6] Their IR spectra suggest that aromatic rings and chelated hydroxyl groups were present, in keeping with such a structure.

The ^{1}H NMR spectrum of the less polar phenylethanone, **2**, $C_{18}H_{24}O_4$, indicated the presence of a chelated and a free hydroxyl group, an unsubstituted aromatic position and an acetyl group. Two singlets, each due to two methyl groups, together with two vinyl proton triplets and two methylene proton doublets in the spectrum showed that two isopentenyl groups were present The chemical shifts of the doublets indicated that while one was benzylic in nature, the other was probably attached to an oxygen atom in an ether linkage The loss of C_5H_9 and C_4H_7 moieties observed in the mass spectrum of **2** gave further evidence for such an arrangement

Acetylation of **2** to the diacetate **4** caused a significant downfield shift (0 12 ppm) of the benzylic isopentenyl CH_2 signals in the ^{1}H NMR spectrum, suggesting that



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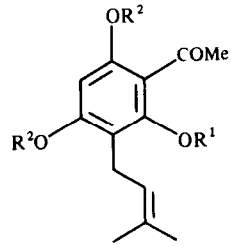
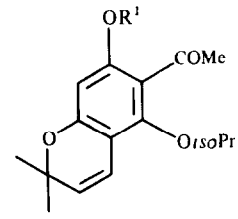


2 R^1 = *iso*Pr, R^2 = H
3 R^1 = Ger, R^2 = H
4 R^1 = *iso*Pr, R^2 = Ac
7 R^1 = Ger, R^2 = Ac



5 R^1 = H
6 R^1 = Ac

this CH_2 group was in close proximity to one of the hydroxyl groups in **2** The corresponding shift of the ether isopentenyl CH_2 was negligible (0 01 ppm)

The phenylethanone therefore has a structure containing two hydroxyl groups, an acetyl group, an isopentenyl group and an *O*-isopentenyl group, occupying five positions in an aromatic ring It did not give a positive Gibb's test [7] indicating that the unsubstituted aromatic position was not *para* to a hydroxyl group This suggested that the isopentenyl group was *para*- to the chelated hydroxyl group while the second hydroxyl group was *para*- to the acetyl group, which itself would be in an *ortho*-position to the *O*-isopentenyl group Its structure was therefore that of 1-[2',4'-dihydroxy-6'-(3''-methyl-2''-butenyloxy)-5'-(3''-methyl-2''-butenyl)]phenylethanone (**2**)

Cyclization of **2** with DDQ gave 6-acetyl-7-hydroxy-5-(3'-methyl-2'-butenyloxy)-2,2-dimethyl-3,4-dihydro-[2*H*]-1-benzopyran (**5**), giving further evidence for this structure. Its ^{1}H NMR spectrum retained the chelated OH proton singlet but a pair of AB doublets replaced the benzylic CH_2 proton signal Cyclization had therefore taken place as would be expected between the non-chelated 4'-OH group and the 5'-isopentenyl group A significant shift in ^{1}H NMR on acetylation of **5** was seen only for the acetyl and aromatic proton signals.

The ^{1}H NMR spectrum of the more polar phenylethanone, **3**, $C_{23}H_{32}O_4$, showed the presence of both a non-chelated and a chelated OH group, an acetyl group and one unsubstituted aromatic position as in **2**

Molecular formula considerations indicated that the remaining substituents on the aromatic ring would be an alkyl group and an *O*-alkyl group The mass spectrum of **3** showed an intense peak at *m/z* 236 corresponding to the loss of a $C_{10}H_{16}$ unit from the molecular ion and a base peak at *m/z* 181 corresponding to a further loss of a C_4H_7 unit The loss of a $C_{10}H_{16}$ rather than a C_9H_{14} fragment indicated that an *O*-geranyl group was present, while cleavage of a C_4H_7 unit suggested that the isopentenyl group was directly attached to the benzene ring

The phenylethanone **3** should have a structure containing two hydroxyl groups, an acetyl group, an isopentenyl group and an O-geranyl group occupying five positions in an aromatic ring A negative Gibb's test indicated that the unsubstituted aromatic position was not *para*- to a hydroxyl group The phenylethanone was therefore 1-[2',4'-dihydroxy-6'-(3'',7''-dimethylocta-2'',6''-dienyloxy)-5'-(3''-methyl-2''-butenyl)]phenylethanone (**3**) Acetylation of **3** gave the diacetate **7**

EXPERIMENTAL

Mps uncorr UV EtOH IR KBr ^{1}H NMR 60 MHz, $CDCl_3$ using TMS as int. standard EIMS 70 eV, direct probe Optical rotations $CHCl_3$ at 25° Prep TLC and MPLC Merck silica gel $PF_{254+366}$ and Kieselgel 60 (230–400 mesh), respectively Petrol 40–60° Identities of compounds were established by mmp, IR and ^{1}H NMR comparisons, unless otherwise stated

Euodia lunu-ankenda was collected from Mooloya Estate, Hewaheta in central Sri Lanka and a voucher specimen has been deposited at the University herbarium

Extraction Dried powdered *E lunu-ankenda* root bark (1 5 kg) was extracted successively with CH_2Cl_2 and MeOH at 27° for two 24 hr periods each Concn of the combined solns at 40° gave 19 2 and 10 1 g of the CH_2Cl_2 and MeOH extracts, respectively

Separation of the basic fraction of the CH_2Cl_2 extract The CH_2Cl_2 extract (18 8 g) was dissolved in Et_2O (500 ml) and washed (× 3) with 2% HCl (400 ml) Concn of the CH_2Cl_2 layer at 40° gave the neutral fraction (16 8 g) The aq layer was washed with Et_2O, neutralized with Na_2CO_3 and extracted with CH_2Cl_2 Concn of the CH_2Cl_2 extract at 40 gave the basic fraction (461 mg)

Chromatography of the basic fraction Prep TLC (cyclohexane–petrol–EtOAc, 9 9 2, 2 developments) gave, after chromatography on silica gel, dictamine as needles (52 mg), from CH_2Cl_2–petrol, mp 129–131 (lit [8] mp 132°) and after prep TLC (CH_2Cl_2), evolitrine as needles (55 mg) from CH_2Cl_2–petrol, mp 111–113° (lit [2] mp 114) The polar bands gave as needles from CH_2Cl_2–petrol γ-fagarine (8 mg), mp 141–143° (lit [8] mp 142°), skimmianine (12 mg), mp 175–177° (lit [8] mp 176°) and kokusaginine (14 mg), mp 167–170° (lit [9] mp 172°)

Chromatography of the neutral fraction The neutral fraction (16 8 g) was chromatographed (MPLC) using petrol–EtOAc–MeOH mixtures for elution

Elution with EtOAc–petrol (1 49) gave elemental sulphur (1 3 g), mp 110–115

Elution with EtOAc–petrol (1 9) gave, by prep TLC (CH_2Cl_2–petrol, 2 3) on recrystallization from MeOH, lupeol (63 mg), mp 214–215°, $[\alpha]_D$ +32 (lit [8] mp 215–216°, $[\alpha]_D$ +26°), identical with authentic lupeol

Elution with EtOAc–petrol (1 4) followed by MPLC using EtOAc–petrol mixtures gave, on prep TLC (toluene–petrol, 3 1) 1-[2',4'-dihydroxy-3' 5'-di(3''-methyl-2''-butenyl)-6'-methoxy)-phenylethanone (**1**) as an yellow oil (42 mg) identical with an authentic sample [5]

Elution with EtOAc petrol (3 7) gave on prep TLC (petrol–EtOAc, 17 3), 1-[2',4'-dihydroxy-6'-(3''-methyl-2''-butenyloxy)-5'-(3''-methyl-2''-butenyl)]phenylethanone (**2**), colourless needles from CH_2Cl_2 (67 mg), mp 73–75°, (HRMS 304 1654 [M]$^+$ Calc for $C_{18}H_{24}O_4$ 304 1674), UV $\lambda_{max}^{CH_2Cl_2}$ nm 288 (log ε 4 70) and 243 (4 35) IR ν_{max} cm^{-1} 3350, 3100 and 1085 ^{1}H NMR δ1 73 and 1 80 (each s, 6H, Me), δ2 66 (s, 3H, COMe), δ3 31 and 4 51 (each *d*, 2H *J* = 7 Hz 1''-H), δ5 21 and 5 44 (each *t*, 1H, *J* = 7 Hz, 2''-H), δ6 00 (*s*, 1H 3'-H), δ8 33 and 14 26 (each s, 1H, D_2O exchangeable, OH) MS *m/z* (rel int) 304 [M]$^+$ (14), 249 (4), 236 (42), 235 (30), 221 (50), 193 (46) and 181 (100), and 1-[2',4'-dihydroxy-6'-(3'' 7''-dimethylocta-2'',6''-dienyloxy)-5'-(3''-methyl-2''-butenyl)]phenylethanone (**3**) as colourless needles from CH_2Cl_2 (81 mg), mp 88–90°, (HRMS 372 2301 [M]$^+$ and 236 1048 [M – $C_{10}H_{16}$]$^+$ Calc for $C_{23}H_{32}O_4$ and $C_{13}H_{16}O_4$ 372 2300 and 236 1049) UV λ_{max} nm 288 (log ε 4 80) and 243 (4 35), IR ν_{max} cm^{-1} 3200, 1665 and 1595, ^{1}H NMR δ1 59 (s, 3H, Me), δ1 65–1 80 (overlapping s, 12H, Me), δ2 08 (*m*, 4H $W_{\frac{1}{2}}$ = 6 Hz, allylic H), δ2 64 (s, 3H, COMe), δ3 31 and 4 52 (each *d*, 2H, *J* = 7 Hz, 1'-H), δ4 98 5 25 (*m*, 2H, $W_{\frac{1}{2}}$ = 14 Hz, vinyl H), δ5 42 (*t*, 1H, *J* = 7 Hz, vinyl H), δ5 98 (s, 1H, 3'-H), δ8 76 and 11 50 (each s, 1H, D_2O exchangeable, OH), MS *m/z* (rel int) 372 [M]$^+$ (9), 317 (2), 236 (64), 221 (48), 193 (47) and 181 (100)

Elution with EtOAc petrol (2 3) gave by prep TLC (CH_2Cl_2–petrol, 1 4) and recrystallization from CH_2Cl_2–petrol, colourless needles of bergapten (61 mg), mp 186–188° (lit [8] mp 188°), identical with an authentic sample

Acetylation of **2** Phenylethanone **2** (39 mg) with Ac_2O–pyridine (1 2, 3 ml) at 27° for 18 hr gave on work-up 1-[2',4'-diacetoxy-6'-(3''-methyl-2''-butenyloxy)-5'-(3''-methyl-2''-butenyl)]phenylethanone (**4**) (33 mg), as a yellow oil, IR ν_{max} cm^{-1} 1755, 1690, 1600 and 1170 ^{1}H NMR δ1 60 1 80 (overlapping *s*, 12H, 3''-Me), δ2 22 and 2 25 (each s, 3H, OAc), δ2 38 (*s*, 3H, COMe), δ3 21 and 4 51 (each *d*, 2H *J* = 7 Hz, 1''-H), δ5 09 and 5 45 (each *t*, 1H *J* = 7 Hz, 2'-H) and δ6 53 (s, 1H, 3'-H) MS *m/z*

(rel int.). 389 $[M+1]^+$ (24), 245 (36), 321 (25), 304 (24), 277 (40), 235 (100), 193 (29) and 181 (40)

Cyclization of **2**. Phenylethanone **2** (57 mg) was refluxed with DDQ (0.2 g) in C_6H_6 (1 ml) for 18 hr The usual work-up followed by prep TLC (petrol–CH_2Cl_2, 2 1) gave 6-acetyl-7-hydroxy-5-(3′-methyl-2′-butenyloxy)-2,2-dimethyl-3,4-dihydro-[2*H*]-1-benzopyran (**5**) (43 mg), as an oil, IR ν_{max} cm^{-1} 1650 and 1590 ^{1}H NMR δ1 50 (*s*, 6H, 2-Me), δ1.76 and 1 83 (each *s*, 3H, Me), δ2 66 (*s*, 3H, COMe), δ4 57 (*d*, 2H, $J = 7$ Hz, OCH_2), δ5 30–5.65 (*m*, 1H, 2′-H), 5.42 (*d*, 1H, $J = 10$ Hz, 3-H), δ6 03 (*s*, 1H, 8-H), δ6.61 (*d*, 1H, $J = 10$ Hz, 4-H) and δ13 80 (*s*, 1H, D_2O exchangeable, OH). MS *m/z* (rel. int) 302 $[M]^+$ (9), 287 (2), 234 (12), 219 (100), 201 (10) and 69 (29).

Acetylation of **5** Benzopyran **5** (40 mg) with Ac_2O–pyridine (1.2, 3 ml) at 27° for 18 hr gave on work-up 6-acetyl-7-acetoxy-5-(3′-methyl-2′-butenyloxy)-2,2-dimethyl-3,4-dihydro-[2*H*]-1-benzopyran (**6**) (32 mg), as an oil, IR ν_{max} cm^{-1} 1760, 1690, 1600 and 1250, ^{1}H NMR· δ1 46 (*s*, 6H, 2-Me), δ1.73 and 1.81 (each *s*, 3H, Me), δ2.25 (*s*, 3H, OAc), δ2 51 (*s*, 3H, Ac), δ4.53 (*d*, 2H, $J = 7$ Hz, OCH_2), δ5 43 (*t*, 1H, $J = 7$ Hz, 2′H), δ5 54 (*d*, 1H, $J = 10$ Hz, 3-H), δ6 17 (*s*, 1H, 8-H) and δ6.65 (*d*, 1H, $J = 10$ Hz, 4-H) MS *m/z* (rel int) 344 $[M]^+$ (18), 301 (61), 286 (18), 242 (31), 218 (100), 200 (23), 118 (10) and 68 (28).

Acetylation of **3** Phenylethanone **3** (22 mg) with Ac_2O–pyridine (1 2, 3 ml) at 27° for 18 hr gave on work-up 1-[2′,4′-diacetoxy-6′-(3″,7″-dimethylocta-2″,6″-dienyloxy)-5′-(3″-methyl-2″-butenyl)]phenylethanone (**7**) (21 mg), as an oil, IR ν_{max} cm^{-1}· 1760, 1690 and 1590 ^{1}H NMR· δ1 62 (*s*, 3H, Me), δ1 68–1 85 (overlapping *s*, 12H, Me), δ2 04–2.18 (*m*, 4H, $W_{\frac{1}{2}}$ = 8 Hz, allylic H), δ2.23 (*s*, 6H, OAc), δ2 65 (s, 3H, COMe), δ3.30 and 4.56 (each *d*, 2H, $J = 7$ Hz, 1″-H), δ4 95–5 65 (*m*, 3H, $W_{\frac{1}{2}}$ = 14 Hz, vinyl H) and δ6.01 (*s*, 1H, 3′-H). MS *m/z* (rel. int.): 456 $[M]^+$ (0 2), 388 (3), 345 (5), 321 (3), 304 (2), 277 (52), 235 (100) and 181 (50).

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