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TWO 3-*C*-METHYLFLAVONE GLYCOSIDES FROM *EUGENIA KURZII**

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Key Word Index—*Eugenia kurzii*, Myrtaceae, flavonoids, 3-*C*-methylapigenin 5-*O*-rhamnoside, 3-*C*-methylluteolin 5-*O*-rhamnoside

Abstract—In addition to sitosterol glucoside and gallic acid, two new flavone glycosides have been isolated from *Eugenia kurzii* (aerial parts) and characterized as 3-*C*-methylapigenin 5-*O*-rhamnoside and 3-*C*-methylluteolin 5-*O*-rhamnoside by spectral analysis and chemical conversion 7,4′-Dimethyl-3-*C*-methylapigenin and 7,3′,4′-trimethyl-3-*C*-methylluteolin were synthesized by modified Baker–Venkataraman transformations and compared with the methyl ethers of the natural aglycones

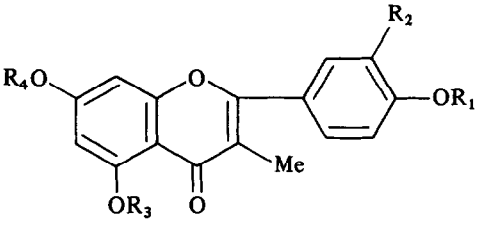
INTRODUCTION

Eugenia kurzii Duthie (syn *E cerasiflora* Kurz) an evergreen tree growing in the eastern Himalayas, Assam and Andman has not been previously chemically investigated A 90% ethanolic extract of the aerial part of the plant collected from Andhra Pradesh showed promising antihypertensive activity The extract was further partitioned into benzene, ethyl acetate, *n*-butanol and water soluble fractions Chromatography of the ethyl acetate fraction yielded two new flavone glycosides whose structural elucidation is dealt with in the present communication

RESULTS AND DISCUSSION

Compound **1**, mp 210°, $C_{22}H_{22}O_9$ (M^+, 430) responded to Fiegel's and Shinoda tests suggesting it to be a hydroxyflavone glycoside Its IR spectrum displayed strong absorption bands ν_{max} cm^{-1} 3500 (OH), 1665 (C=O) and the usual bands for a benzenoid system The UV spectrum of the glycoside had a more pronounced band I 315 nm than the band II 260 nm. There was no change in the UV maxima on addition of aluminium chloride and aluminium chloride–hydrochloric acid which eliminated the possibility of an *o*-dihydroxy or free hydroxy groups at C-5 and C-3 The hydroxy groups at C-4′ and C-7 were confirmed by observing bathochromic shifts with sodium methoxide of 50 and 10 nm in bands I and II, respectively [1]

The glycoside on acetylation formed a crystalline pentaacetate **3**, mp 130° which had a sharp doublet at δ 0 8 (J = 6 0 Hz), a distinguishing feature for the rhamnosyl methyl together with another doublet for H-1″ at 4 42 (J = 2 0 Hz) and other sugar protons which resonated between 4.6 and 5 1 A singlet at δ 1 8 integrated for one methyl group while the five acetyl singlets appeared at 1 84, 1 88, 2 08, 2 20 and 2 28 There were resonances for aromatic protons at δ 6 96 and 7 15 (J = 2 5 Hz) in the AB system and at 7 5, 7 8 (J = 10 0 Hz) in the A_2B_2 system indicating the *p*-substitution in ring B and disubstitution in ring A [2]



1 $R_1 = R_2 = R_4 = H$, R_3 = Rha
2 $R_1 = R_4 = H$, $R_2 = OH$, R_3 = Rha
3 $R_1 = R_4 = Ac$, $R_2 = H$, R_3 = Rha(Ac)$_3$
4 $R_1 = R_2 = R_3 = R_4 = H$
5 $R_1 = R_3 = R_4 = Ac$, $R_2 = H$
6 $R_1 = R_4 = Me$, $R_3 = R_2 = H$
7 $R_1 = R_4 = Ac$, $R_2 = OAc$, R_3 = Rha(Ac)$_3$
8 $R_1 = R_3 = R_4 = H$, $R_2 = OH$
9 $R_1 = R_3 = R_4 = Ac$, $R_2 = OAc$
10 $R_1 = R_4 = Me$, $R_2 = OMe$, $R_3 = H$
11 $R_1 = Me$, $R_2 = R_3 = R_4 = H$
12 $R_1 = Me$, $R_2 = OMe$, $R_3 = R_4 = H$

Acid hydrolysis of **1** yielded rhamnose (PC) and an aglycone **4**, mp 215°, $C_{16}H_{12}O_5$ (M^+ 284) which had hydroxyl and chelated carbonyl functionalities at IR ν_{max} cm^{-1} 3450 and 1645, respectively Its UV spectrum had λ_{max}^{MeOH} nm 265, 295 and 330 A bathochromic shift of 45 nm with aluminium chloride and aluminium chloride–hydrochloric acid suggested the presence of a free hydroxy at C-5 in the aglycone The other shifts with different diagnostic reagents were similar to those observed for the parent glycoside The aglycone formed a triacetate **5**, mp 170° whose ^{1}H NMR spectrum showed a signal for a methyl group at δ 1 8, and for three MeCO groups at 2 33, 2 36 and 2 47 Aromatic protons of ring A appeared at δ 6 51, 6 74 (2H, *dd*, J = 2 5 Hz) and ring B

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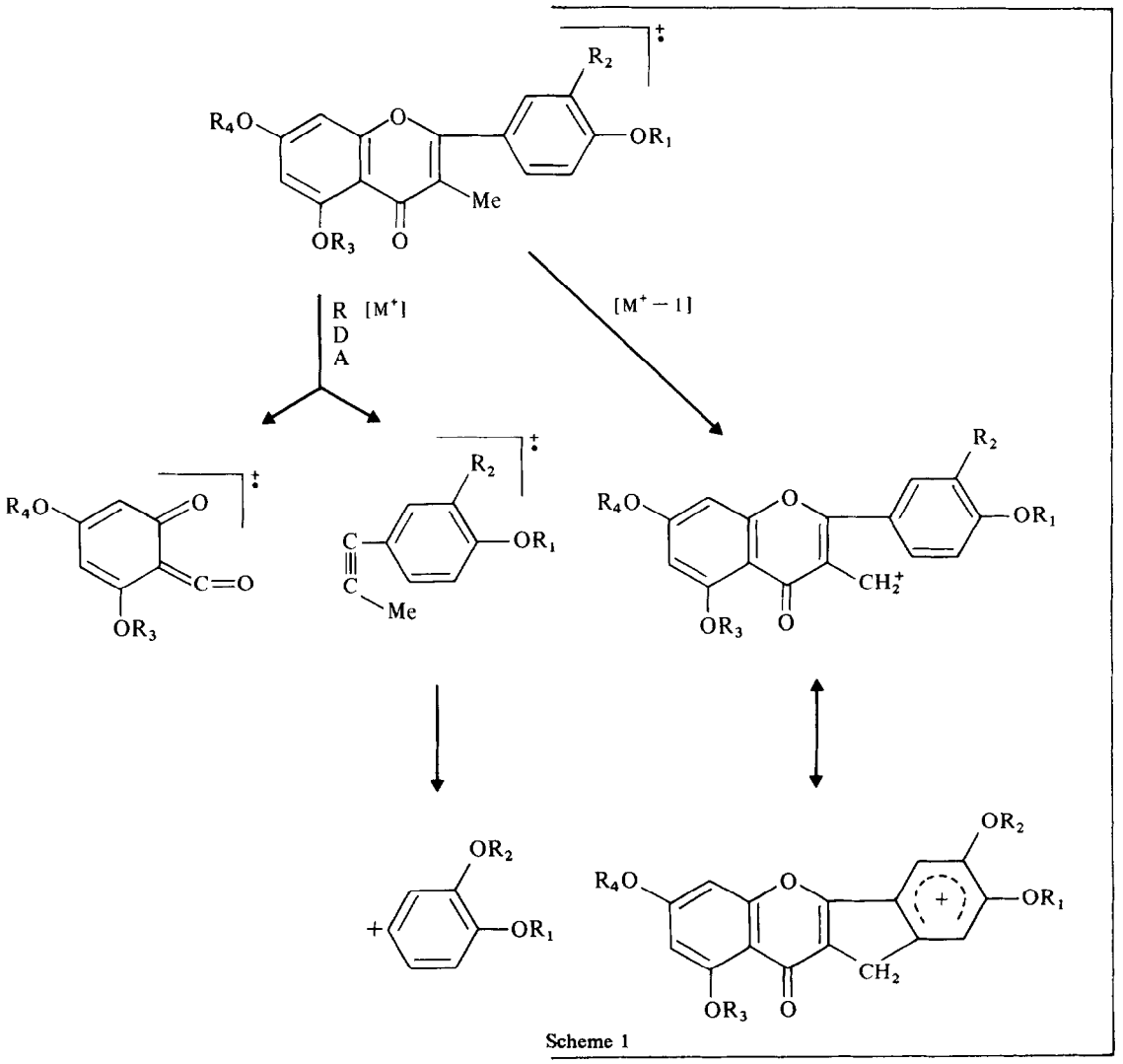
protons at 7 45, 7 75 (4H, *dd*, $J = 10.0$ Hz) These data thus confirmed the three free hydroxyl functions in the aglycone Compound **4** gave a dimethyl ether (**6**), mp 200° having IR bands at ν_{max} cm^{-1} 3415 (OH), 1640 (C=O) and 1590, 830 and 780 (aromatic) Its ^{1}H NMR spectrum had a sharp singlet at δ 1 8 for a methyl function, singlets at 3 8, 4 0 for two methoxyls, 6 5, 7 5 A_2B_2 system and 6 45, 6 17 AB system for aromatic protons

This proved the structure of the aglycone as 5, 7, 4'-trihydroxy-3-methylflavone (**4**), which received further support from its mass fragmentation pattern The mass spectrum had ion peaks at *m/z* (rel int) 284 $[M]^+$ (30), 283 (60), 282 (21 1), 121 (21) and the retro-Diels–Alder fission gave rise to ion peaks at *m/z* 152 (34 3), 132 (15 3) and 93 (14) [3], thus confirming the position of the methyl at C-3 in the molecule (Scheme 1)

acetate–boric acid reagent A bathochromic shift of 11 nm in band II with fused sodium acetate indicated a free hydroxyl group at C-7 in **2**

Acid hydrolysis of **2** yielded rhamnose (PC) and an aglycone, **8**, mp 210° The UV spectrum with different shift reagents was similar to that of luteolin The aglycone formed a tetra-acetate, **9**, mp 180°, $C_{24}H_{20}O_{10}$ (M^+ 342) and a trimethyl ether, **10**, mp 205° with diazomethane All the data showed that **2** had a similar structure to **1** but with an additional hydroxy group at C-3' in the B ring Thus, the second glycoside was identified as 7, 3', 4'-trihydroxy-3-*C*-methylflavone 5-*O*-rhamnoside or 3-*C*-methylluteolin 5-*O*-rhamnoside (**2**)

This is the first report of flavone glycosides having a C-3 methyl function from a natural source However, the synthesis of flavones with C-3 methyl functions are



Scheme 1

The second compound (**2**) mp 225° analysed for $C_{22}H_{22}O_{10}$ (M^+, 446) and responded to the colour reactions for flavone glycoside The UV λ_{max}^{MeOH} nm 329, 292 sh, 260 showed no change with aluminium chloride–hydrochloric acid indicating the absence of free hydroxyls at C-5 or C-3 The presence of an *ortho*-dihydroxy group in the ring B was confirmed by a bathochromic shift of 21 nm in band I with sodium

reported in the literature [4–6] The structures were confirmed by the synthesis of the methyl ethers of the aglycones by modified Baker–Venkataraman transformation [4] Condensation of phloropropiophenone with anisic anhydride and veratroyl chloride in the presence of potassium carbonate in acetone afforded 4'-methoxy-5,7-dihydroxy-3-*C*-methylflavone (**11**) and 3',4'-dimethoxy-5,7-dihydroxy-3-*C*-methylflavone (**12**), respectively

Methylation of **11** and **12** with diazomethane afforded 7,4′-dimethyl-3-*C*-methylapigenin and 7, 3′, 4′-trimethyl-3-*C*-methylluteolin analogous to **6** and **10** (mp, mmp, TLC, ^{1}H NMR, MS) obtained from aglycones isolated and characterized

EXPERIMENTAL

Uncorr capillary mps are reported IR, UV and 60 MHz ^{1}H NMR spectra were taken in KBr, MeOH and $CDCl_3$ (unless otherwise stated) with TMS as an int standard The TLC was performed on Si gel G plates and flavones visualized by ceric sulphate spray The known compounds were identified by comparing the mp, mmp, IR spectrum and by co-TLC with those of authentic samples

The air-dried plant material [*E kurzii* (aerial part, 10 kg)] collected from Mannanur (Andhra Pradesh), voucher specimen preserved in CDRI, was extracted with 90% EtOH The EtOH extract was then fractionated with C_6H_6 and EtOAc, respectively The EtOAc extract (50 g) was chromatographed over Si gel (1 5 kg) Fractions eluted with $CHCl_3$–MeOH (4 1) gave a residue (5 5 g) containing substances **1** and **2** with streaking material This residue was filtered through a polyamide column Elution with H_2O satd EtOAc–MeOH (19 1) gave a yellow residue (1 35 g) containing both compounds Both **1** and **2** were finally purified by TLC EtOAc–MeOH–H_2O, 8 1 1, R_f 0 4 and 0 3, respectively)

Substance **1** Crystallized from MeOH, mp 210° Found C, 61 80, H, 5 18 $C_{22}H_{22}O_9$ requires C, 61 39, H, 5 11%

Acetate of **1** Acetylation of **1** with C_5H_5N–Ac_2O at room temp yielded a crude viscous mass Chromatography of the product gave a pure acetate, **3**, mp 130° IR ν_{max}^{KBr} cm^{-1} 1770 (ester) Found C, 60 18, H, 5 15 $C_{32}H_{32}O_{14}$ requires C, 60 00, H, 5 0%

Hydrolysis of **1** Substance **1** (100 mg) in 2 ml EtOH on hydrolysis with 6% HCl (20 ml) afforded the aglycone **4**, mp 215° ^{1}H NMR (DMSO-d_6) δ1 85 (*s*, Me), 6 25, 6 5 (2H, *dd*, *J* = 2 5 Hz), 6 95, 8 13 (4H, *dd*, *J* = 10 0 Hz) Found C, 64 12, H, 4 04 $C_{16}H_{12}O_5$ requires C, 64 04, H, 4 22%

Acetate of 3-C-*methylapigenin* (**5**) Mp 170° IR ν_{max}^{KBr} cm^{-1} 1745 (ester) Found C, 64 0, H, 4 10 $C_{22}H_{18}O_8$ requires C, 64 39, H, 4 39%

Methyl ether of 3-C-*methylapigenin* (**6**) 3-Methylapigenin was methylated with ethereal CH_2N_2, mp 200° Found C, 68 99, H, 5 0 $C_{18}H_{16}O_5$ requires C, 69 23, H, 5 12%

Substance **2** Crystallized from MeOH, mp 225° IR ν_{max}^{KBr} cm^{-1} 1665 (carbonyl) Found C, 59 02, H, 4 80 $C_{22}H_{22}O_{10}$ requires C, 59 18, H, 4 01%

Acetate of **2** Mp 150° IR ν_{max}^{KBr} cm^{-1} 1770, 1750 (ester) ^{1}H NMR δ0 83 (3 H, *d*, Me, *J* = 6 0 Hz), 1 8 (3H, *s*, Me), 1 9, 1 98, 2 1 (9H, *s*, 3 OCOMe), 2 2 (6H, *s*, 2 OCOMe), 2 35 (3H, *s*, OCOMe), 4 8–5 3 (4H, *m*), 5 7 (1H, *d*, *J* = 2 0 Hz), 6 5, 6 65 (2H, *dd*, *J* = 2 5 Hz), 7 3 (1H, *d*, *J* = 10 0 Hz), 7 7 (2H, *dd*, *J* = 10 0 and 2 5 Hz) Found C, 58 40; H, 3 63 $C_{34}H_{24}O_{16}$ requires C, 58 45, H, 3 58%

Hydrolysis of **2** Glycoside was hydrolysed with 6% methanolic HCl at 100° for 5 hr Aglycone **8** was crystallized from MeOH, mp 210° UV λ_{max}^{MeOH} 340, 296 sh, 267, + $AlCl_3$ 420, 328, 300 sh, + $AlCl_3$–HCl 390, 300, 273, + NaOMe 400, 324, 302 sh, 279, + NaOAc 398, 310, 300 sh, 278, + NaOAc–H_3BO_3 373, 301 sh, 269 nm IR ν_{max}^{KBr} cm^{-1} 3450 (OH), 1640, 1610 (flavone) ^{1}H NMR (DMSO-d_6) δ1 9 (*s*, Me), 6 0 and 6 15 (2H, *dd*, *J* = 2 5 Hz), 6 5 (1H, *d*, *J* = 10 0 Hz) and 7 75 (2H, *t*, *J* = 10 0 and 2 5 Hz) MS *m/z* (rel int) 300 [M]$^+$ (58), 299 (17 3), 152 (34 0), 148 (19 0), 137 (30 2), 109 (14 1), 44 (100%) Found C, 64 30; H, 4 05 $C_{16}H_{12}O_6$ requires C, 64 0, H, 4 0%

Acetate of 3-C-*methylluteolin* (**9**) Mp 180° IR ν_{max}^{KBr} cm^{-1} 1770, 1750 (ester) ^{1}H NMR ($CDCl_3$) δ1 8 (3H, *s*, Me), 2 28 (6H, *s*, 2COMe), 2 35 and 2 47 (6H, each *s*, 2COMe), 6 5 and 6 65 (2H, *dd*, *J* = 2 5 Hz), 7 1 (1H, *d*, *J* = 10 0 Hz) and 7 93 (2H, *dd*, *J* = 10 0 and 2 5 Hz)

Methyl ether of 3-C-*methylluteolin* The aglycone on methylation with diazomethane gave **10**, mp 205° IR ν_{max}^{KBr} cm^{-1} 1640, 1610 (flavone) ^{1}H NMR δ1 8 (3H, *s*, Me), 3 88 (6H, *s*, 2OMe), 3 95 (3H, *s*, OMe), 5 77 and 6 09 (2H, *dd*, *J* = 2 5 Hz), 6 8 (1H, *d*, *J* = 10 0 Hz) and 7 5 (2H, *dd*, *J* = 10 0 and 2 5 Hz) MS *m/z* 342 [M]$^+$

Synthesis of 4′-*methoxy*-5,7-*dihydroxy*-3-C-*methylflavone* (**11**) A mixture of phloropropiophenone (500 mg) (prepared from phloroglucinol, propionitrile in the presence of $ZnCl_2$ by passing dry HCl gas) [7] and anisic anhydride (3 eq) in dry Me_2CO (30 ml) in the presence of dry K_2CO_3 (3 0 g) was refluxed for 15 hr The Me_2CO was removed under vacuum and residue dissolved in H_2O The soln was neutralized with cold dil HCl and extracted with EtOAc The EtOAc layer was washed, dried and concd The residue was chromatographed over Si gel Elution with C_6H_6–EtOAc (5 1) afforded **11** (300 mg), mp 226° (Me_2CO) IR ν_{max}^{KBr} cm^{-1} 3500 (OH), 1660 (C=O), 1610, 1500, 850 (aromatic) ^{1}H NMR (DMSO-d_6) δ1 98 (*s*, Me), 3 77 (*s*, OMe), 6 15 and 6 29 (2H, *dd*, *J* = 3 0 Hz), 6 85 and 7 86 (4H, *dd*, *J* = 10 0 Hz) MS *m/z* (rel int) 298 [M]$^+$ (40 4), 297 (60 2), 153 (10 9), 152 (88 4), 147 (6 8), 135 (100) and 107 (11 7)

Synthesis of 3′, 4′-*dimethoxy*-5,7-*dihydroxy*-3-C-*methylflavone* (**12**) Phloropropionphenone (500 mg), veratroyl chloride (2 0 g), K_2CO_3 (3 0 g) in dry Me_2CO (30 ml) were refluxed for 30 hr After work-up the residue obtained was chromatographed over Si gel Elution with $CHCl_3$–Me_2CO (95 5) afforded **12** (270 mg), mp 250° (Me_2CO) IR ν_{max}^{KBr} cm^{-1} 3020 (OH), 1630 (C=O), 1605, 1580, 1500, 850 and 830 (aromatic) ^{1}H NMR (DMSO-d_6) δ1 9 (*s*, Me), 3 78, 3 82 (*s*, 2 OMe), 6 16 and 6 29 (2H, *dd*, *J* = 3 0 Hz), 6 35 (1H, *d*, *J* = 10 0 Hz), 7 52 (2H, *t*, *J* = 10 0 and 2 5 Hz) MS *m/z* (rel int) 328 [M]$^+$ (86 7), 327 (100), 313 (18 8), 297 (21 2), 153 (7 3), 150 (14 0) and 121 (8 7)

Methylation of **11** *and* **12** 50 mg of each were methylated with ethereal CH_2N_2 at 0° for 12 hr Removal of Et_2O afforded 4′, 7-dimethyl-3-*C*-methylapigenin (**6**) and 3′,4′,7-trimethyl-3-*C*-methylluteolin (**10**)

The hydrolysate was neutralized with $BaCO_3$, filtered and concd The sugar was identified as rhamnose with an authentic sample through PC using Whatman No 1 paper, *n*-BuOH–HOAc–H_2O (4 1 5) as solvent, and aniline phthalate as spraying reagent

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