posed for eugenol and cinnamic aldehyde is similar to that suggested by Manitto *et al.* [2–4] for *Ocimum basilicum*.

EXPERIMENTAL

Materials and methods. Fresh cinnamon cuttings were obtained from Royal Botanic Gardens, Sydney. They were divided into 5 lots each of 25 ± 2 g. The stems of the selected cuttings (*ca* 15–20 cm long), each with 5–8 attached leaves were then recut. The cut ends were immediately immersed in glass vials containing the solns with labelled substrate. The substrate solns were 10 μCi of DL-phenyl-[1-^{14}C]-alanine, DL-phenyl-[3-^{14}C]-alanine, 5 μCi of DL-phenyl-[2-^{14}C]-alanine, 10 μCi of L-[^{14}C-Me]-methionine and 10 μCi of L-[^{14}C-Me]-methionine with 7 μCi DL-phenyl-[3-^{14}C]-alanine, each lot with 4 mmol and 1 mg glucose. The total vol. of soln in each vial was 0.1 ml. The cuttings were held in the glass house under normal daylight condition, at a constant temp. (25 ± 1°). Most of the substrate solns were adsorbed in about 30 min; after which the cuttings were maintained on H_2O.

Extraction and isolation procedure. After 5 hr each lot was removed, cut into small pieces and steam distilled immediately. The radioactivity of the distilled oil was determined using toluene based scintillation soln and a scintillation counter.

Isolation of eugenol and cinnamic aldehyde. These two compounds were isolated by preparative GLC on a (3 × 6.4 m o.d.) glass column of 20% Carbowax 20 M on Gas-chrom Q (80–100 mesh). The operating conditions were: column temp. 75–235° at 2°/min; injector 210°; detector 235°; carrier gas N_2 45 ml/min; stream splitter 1 :10; sample size 30 μl. The eluant samples were collected in a glass tube (7 × 0.25 i.d.) packed with Si gel 50–200 mesh with either end plugged with glass wool. The relative abundance of each compound was calculated by peak areas. The radioactivity in eugenol and cinnamic aldehyde was measured using toluene based scintillation soln (10 ml) containing 40% Cab-o-sil.

Isolation and degradation of labelled eugenol. Eugenol was isolated from the distilled leaf oil by method of ref. [1]. Labelled cinnamon leaf oil (460 μl) was dissolved in Et_2O (5 ml) and extracted by shaking with 10% KOH (3 × 5 ml). Eugenol was regenerated by acidifying with excess H_2SO_4, extracted with Et_2O and concd under a gentle stream of N_2, when its radioactivity was determined. GLC showed only one peak corresponding to eugenol. Eugenol labelled with L-[^{14}C-Me]-methionine was subjected to degradation to homoveratric acid by a modification of the method described in ref. [3]. Labelled eugenol (225 μl) mixed with inactive eugenol (25 μl) and methylated by refluxing for 5 hr with Me_2SO_4 (0.7 ml) and 10% KOH (0.7 ml) in dioxane (12.5 ml). The methyleugenol was extracted with ether, dried and concd over a gentle stream of N_2. Methyleugenol was degraded to homoveratric acid (3,4-dimethoxyphenylacetic acid) as follows. A soln of methyleugenol (100 μl), $KMnO_4$ (2.5 g) and K_2CO_3 (0.8 g) in 100 ml H_2O was refluxed for 3 hr, cooled and filtered. Homoveratric acid was extracted with $CHCl_3$ (25 ml × 2) and Et_2O (25 ml). The combined soln was evapd and the homoveratric acid was recrystallized from hot H_2O to constant mp (81.5–82.5°). Its radioactivity was determined using toluene based scintillation solution.

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REFERENCES

1. Wijesekera, R. O. B., Jayewardena, A. L. and Rajapakse, L. S. (1974) *J. Sci. Food Agric.* **25**, 1211.
2. Cannonica, L. Manitto, P., Monti, D. and Sanchez, A. M. (1971) *Chem. Commun.* 1108.
3. Manitto, P., Monti, D. and Gramatica, P. (1974) *J. Chem. Soc. Perkin I* 1727
4. Manitto, P., Gramatica, P. and Monti, D. (1975) *J. Chem. Soc. Perkin I* 1548.
5. Klischies, M., Stockigt, J. and Zenk, M. H. (1975) *Chem. Commun.* 879.
6. Tressl, R. and Drawert, F. (1973) *J. Agric. Fd Chem.* **21**, 560.

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TWO NEW COUMARINS FROM *RUTA PINNATA**

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Key Word Index—*Rupta pinnata*; Rutaceae; coumarins; 6(2′-keto-3′-methyl)butyl-7-methoxycoumarin; tamarin; 6(2-hydroxy-3′-ethoxy)-butyl-7-methoxycoumarin.

INTRODUCTION

Ruta pinnata L.fil, endemic to the Canary Islands, is extremely rich in coumarins, of which we have already isolated more than forty, nine being reported for the first time [1–4]. We have already reported on the coumarins from the leaves of this plant [4–6]; this paper deals with secondary coumarins from the same source.

RESULTS AND DISCUSSION

From the alcohol extract of the leaves of *Ruta pinnata*,

*Part 34 in the series 'New Sources of Natural Coumarins'. For part 33, see: González, A. G., Cardona, R. J., Diaz Chico, E., Dorta, H. L. and Rodriguez Luis, F. (1977) *Anales Quim.* in press.

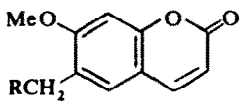
the coumarins already described [4–6] were isolated, together with benahorin, byakangelicin, furopinnarin, heraclenol, luvangetin, oxypeucedanin hydrate, pangelin, pinnarin, sabandinin, tamnosin, xanthotoxin and xanthyletin, previously obtained from other parts of the same plant and characterized by their constants and spectroscopic data. Coumarin **1** has not been found before as a natural product and **2** and **6** are hitherto unknown.

Coumarin 1

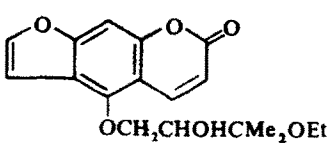
Mp 89–90°, $C_{15}H_{16}O_4$, *m/e* M^+ 260, with significant fragments at 217 [$M^+ - CH(CH_2)_2$] and 189 (base peak) [$M^+ - CH_2-CO-CHMe_2$]. The UV spectrum shows it to be a simple coumarin with alkylation at C-6 and alkoxylation at C-7. Its NMR (60 MHz, $CDCl_3$, τ-scale) has signals at 2.38 and 3.75 (1H each, *d*, J = 10 Hz; H-4 and H-3); 2.79 and 3.21 (1H each, *s*; H-5 and H-8) plus those corresponding to the group $-CH_2-CO-CHMe_2$. These data are the same as those for 6(2'-keto-3'-methyl)-butyl-7-methoxycoumarin (**1**), synthesized by King *et al.* [7] from 2',3'-epoxysuberosin.

Another coumarin isolated from the leaves of *Ruta pinnata* is **2**: mp 112–113°, $C_{19}H_{16}O_4$, *m/e* M^+ 260, major peaks, 189 (base peak) ($M^+ - CHOHCMe = CH_2$), 175 ($M^+ - CH_2CHOHCMe = CH_2$). Its UV was consonant with that of a 7-methoxycoumarin and an OH was plainly visible in the IR spectrum (ν_{max}^{nujol} 3325 and 3225 cm^{-1}). Signals appeared in its NMR at: 2.39 and 3.80 (1H each, *d*, J = 9.5 Hz; H-4 and H-3), 2.70 and 3.23 (1H each, *s*; H-5 and H-8); it also had the signals typical for the $CH_2CHOHCMe = CH_2$ grouping. By oxidation (CrO_3/Py), **2** formed ketone **5** and by acetylation, the monoacetate **4**, mp 76–77°, giving the dihydro derivative **3** by hydrogenation. When **2** was heated in acetic acid with a few drops of sulphuric acid, it isomerized to ketone **1**. We suggest the trivial name, tamarin, for this new coumarin.

From the alcoholic leaf extract an oily product **6** which could not be crystallized was isolated: $C_{17}H_{22}O_5$, *m/e* M^+ 306, significant peaks, 261 ($M^+ - OEt$), 219 ($M^+ - CMe_2OEt$), 189 (base peak) ($M^+ - CHOHCMe_2OEt$). The UV spectrum of **6** was superimposable on those of **1** and **2** while its IR revealed OH absorption (ν_{max}^{film} 3460 cm^{-1}). Its NMR (90 MHz, $CDCl_3$, τ-scale) showed absorption bands at 2.35 and 3.76 (1H each, *d*, J = 9.5 Hz; H-4 and H-5), 2.62 and 3.20 (1H each, *s*; H-5 and H-8) and signals characteristic of the group $-CH_2-CHOH-CMe_2OEt$. The presence of the —CHOH— group was confirmed by the formation of an acetate **7** and by oxidation (CrO_3–Py) to the ketone **8**.



1, R = $COCHMe_2$
2, R = $CHOHCMe = CH_2$
3, R = $CHOHCHMe_2$
4, R = $CHOAcCMe = CH_2$
5, R = $COCMe = CH_2$
6, R = $CHOHCMe_2OEt$
7, R = $CHOAcCMe_2OEt$
8, R = $COCMe_2OEt$



9

The spectral data obtained for **6** and for its derivatives are in accordance with the structure 6(2-hydroxy-3'-ethoxy)butyl-7-methoxycoumarin, which is a new coumarin. It could be formed as an artifact from **2** during the extraction process.

From the alcoholic extract of the leaves of *Ruta pinnata* the *O*-tri-ethyl-hydrate of oxypeucedanin **9**, a coumarin in the fruits of *Ruta oreojasme* Webb [8] was also isolated, as was the lignan, savinin.

EXPERIMENTAL

The mp's are uncorr. UV spectra were taken in EtOH; IR spectra on a PE 257 and NMR on a PE-R32, using TMS as int. stand.

Extraction. 5 kg dried leaves of wild *Ruta pinnata*, gathered in the region of Bajamar (Tenerife) were extracted under reflux with EtOH. When the extract had been concd, the sticky residue was rinsed in H_2O vapour. It was extracted again by refluxing with petrol and then with C_6H_6. After the C_6H_6 had been removed, 103 g of a dark substance were left (C_6H_6 extract). The liquid from the rinse was cleaned in $CHCl_3$, yielding 22 g of a semi-solid reddish product ($CHCl_3$ extract).

Separation and characterization of the products. C_6H_6 extract: this was chromatographed on a column of Al_2O_3 (Merck) of II–III activity and eluted with petrol–C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$, $CHCl_3$–EtOH and EtOH. This process yielded predominantly coumarin mixtures later separated on Al_2O_3 or Si gel. The following coumarins were isolated. simple–pinnarin, sabandin, sabandinin and sabandinol; furocoumarins—benahorin, bergapten, furopinnarin, isopimpinellin, oxypeucedanin hydrate, pangelin and xanthotoxin; pyranocoumarins—luvangetin and zanthyletin; the bicoumarin, thamnosin and the lignan, savinin. All products were identified by their constants, spectral data (IR, UV, NMR and MS) and by TLC and PC of these products and samples of known substances. From the benzene extract, **1**, **2**, **6** and **9** were also isolated. $CHCl_3$ extract: the 22 g of the $CHCl_3$ extract were subjected to chromatography on a column of 450 g of activated (IV) [10% H_2O] Al_2O_3 and the following substances were isolated: byakangelicin, 2',3'-dihydroxy-2',3'-dihydrosuberosin, heraclenol, oxypeucedanin hydrate and sabandinol, further identified by their constants, by spectroscopy and by comparative chromatography.

6(2'-Keto-3'-methyl)butyl-7-methoxy-coumarin (**1**). Blue fluorescence in UV. Mp 88–89°; IR ν_{max}^{nujol} 1725, 1628, 1568, 821 cm^{-1}; UV λ_{max} 327, 291 (inf), 251.5, 240 (inf), 221 and 240 nm (log ε, 3.88, 3.62, 3.39, 3.50, 3.96 and 4.14); NMR ($CDCl_3$, 60 MHz) 6.13 (3H, *s*; MeO), 6.23 (2H, *s*; $C\underline{H}_2$—CO), 7.27 [1H, *m*, J = 7Hz; $C\underline{H}Me_2$] and 8.87 (6H, *d*, J = 7Hz; *gem*-dimethyl).

6(2'-Hydroxy-3'-methyl)buten-3-yl-7-methoxy-coumarin (**2**). Blue fluorescence in UV. Mp 112–113°; IR ν_{max}^{nujol} cm^{-1} 3325, 3225, 1619, 1564, 902, 821; UV λ_{max} nm 331, 300, 253, 242 (inf), 223 and 207 (log ε, 3.91, 3.65, 3.47, 3.58, 4.02 and 4.11); NMR ($CDCl_3$, 90 MHz) 5.06 (1H, apparently a *br s*; $=CH_2$), 5.17 (1H, *m*; $=CH_2$), 5.67 (1H, *m*; $CH_2-C\underline{H}$), 6.09 (3H, *s*; MeO), 7.15 (2H, *m*; $C\underline{H}_2$—CH), 7.80 (OH), 8.18 (3H, *s*; =C—Me). Found: C, 69.23; H, 6.18. $C_{15}H_{16}O_4$ requires: C, 69.18; H, 6.19.

6(2'-Hydroxy-3'-methyl)butyl-7-methoxy coumarin (**3**). 50 mg **2** in EtOH were hydrogenated under pressure at 20° with 1% Pd-C as catalyst. A solid was formed which crystallized from C_6H_6: mp 132–133°; IR ν_{max}^{nujol} cm^{-1} 3360, 3320, 1733, 1629, 1565 and 819; UV λ_{max} nm 331, 298 (inf), 252, 241 (inf), 222 and 205 (log ε, 3.86, 3.60, 3.42, 3.50, 3.96 and 4.12); NMR ($CDCl_3$, 60 MHz) 3.39 (1H, *d*, J = 10 Hz; H-4), 2.72 (1H, *s*; H-5), 3.22 (1H, *s*; H-8), 3.79 (1H, *d*, J = 10 Hz), 6.16 (3H, *s*; MeO), 6–7.5 (3H, *m*; $C\underline{H}_2$—$C\underline{H}$), 8.20 (1H, broad-based *s*; OH), 9.07 (6H, *d*, J = 7 Hz; *gem*-dimethyl); MS, M^+ 262 (4, $C_{15}H_{18}O_4$ requires

262), significant peaks 219 [2, M^+ —$CH(CH_2)_2$], 190 (base peak) [M^+ —$C(OH)CHMe_2$].

6(2'-Acetyl-3'-methyl)buten-3-yl-7-methoxy coumarin (**6**). **2** when treated with Ac_2O–Py formed an acetate (**4**): mp 76–77°; MS *m/e* M^+ 302 (6, $C_{17}H_{18}O_5$ requires 302), *m/e* 242 (36, M^+ —MeCOOH), 190 (60, M^+. C(OCOMe)C$\leqslant^{Me}_{CH_2}$), 189 [base peak M^+ —C(H) (OCOMe)C$\leqslant^{Me}_{CH_2}$], and 43 (28, $MeCO^+$); IR γ_{max}^{nujol} cm^{-1} 1730, 1620, 1515, 1240 (MeCO), 1132 (MeCO), 900 (=CH_2), 821; UV λ^{max} nm 328, 295, 251, 241 (inf), 221 and 204 (log ε, 3.85, 3.66, 3.56, 4.01, 4.19); NMR ($CDCl_3$, 60 MHz) 2.40 (1H, *d*, *J* = 10 Hz; H-4), 2.79 (1H, *s*; H-5), 3.21 (1H, *s*; H-8), 3.76 (1H, *d*, *J* = 10 Hz; H-3), 4.56 (1H, *m*; CH_2—CH), 5.13 (2H, *s*; =CH_2), 6.12 (3H, *s*; MeO), 7.05 (2H, *m*; CH_2—CH), 8.08 (3H, *s*; MeCO), 8.25 (3H, *d*, *J* = 1.3 Hz; =C—Me).

6(2'-Keto-3'-methyl)buten-3-yl-7-methoxy coumarin (**5**). **2** was oxidized with CrO_3–Py to form a product (**5**) which gave a blue flourescence in UV: NMR ($CDCl_3$ 90 MHz) 2.32 (1H, *d*, *J* = 10 Hz; H-4), 2.75 (1H, *s*; H-5), 3.12 (1H, *s*; H-8), 3.70 (1H, *d*, *J* = 10 Hz; H-3), 4.85 (2H, *m*; =CH_2), 5.96 (3H, *s*; MeO), 6.12 (2H, *s*; CH—CO) and 7.95 (3H, *s*; =C—Me).

6(2'-Hydroxy-3'-methyl-3'-ethoxy)butyl-7-methoxycoumarin (**6**). This was obtained as a viscous oil which would not crystallize. $C_{17}H_{22}O_5$. MS, M^+ 306 (37, $C_{17}H_{22}O_5$ requires 306), significant peaks, 288 (5, M^+ —H_2O), 220 (31, M^+ —$C_5H_{10}O$), 190 [M^+ —$C(OH)CMe_2OEt$] and 189, base peak. IR ν_{max}^{film} cm^{-1} 3460, 1720, 1620, 1562 and 824. UV λ_{max} nm 332, 300 (inf), 252 (ing) 242 (inf) 222 and 207 (log ε, 3.85, 3.54, 3.62, 4.01 and 4.15), NMR ($CDCl_3$, 90 MHz) shows signals at 2.35 (1H, *d*, *J* = 9.5 Hz; H-4), 262 (1H, *s*; H-5), 3.20 (1H, *s*; H-8), 3.76 (1H, *d*, *J* = 9.5 Hz; H-3), 5.8–7.65 (3H, *m*; —CH_2CH), 6.09 (3H, *s*; MeO), 6.52 (2H, *q*, J = 7.Hz; OCH_2Me), 8.25 (1H, *br s*; OH), 8.75 (6H, *s*; *gem*-dimethyl) and 8.82 (3H, *t*, *J* = 7 Hz; OCH_2CH_3). **6** with Ac_2O–Py forms the oily acetate **7**, $C_{19}H_{24}O_6$: MS *m/e* M^+ 348 ($C_{19}H_{24}O_6$ requires 348), significant peaks 288 (48 M^+ —MeCOOH), 273 (71, M^+ —MeCOOH—Me), 243 (31, M^+ —MeCOOH—OEt) and 189, base peak. IR, ν_{max}^{film} cm^{-1} 1725, 1621, 1580 and 827. UV, λ_{max} 328, 300 (inf), 250 (inf), 241 (inf), 220 (inf) and 206 (log ε 3.87, 3.71, 3.62, 3.67, 4.04 and 4.15). NMR ($CDCl_3$ 60 MHz), 2.41 (1H, *d*, *J* = 10 Hz; H-4), 2.82 (1H, *s*; H-5), 3.25 (1H, *s*; H-8), 3.79 (1H, *d*, *J* = 10 Hz; H-3), 4.75 (1H, *m*; CH_2—CH), 5.84–7.30 (2H, *m*; CH_2—CH), 6.13 (1H, *s*; MeO), 6.50 (2H, *q*, *J* = 7 Hz; —CH_2—Me), 8.20 (3H, *s*; CH_3—CO), 8.83 (6H, *s*; *gem*-dimethyl) and 8.87 (3H, *t*, *J* = 7 Hz; OCH_2—CH_3). **6** was oxidised with CrO_3–Py yielding an oil, 6(2'-keto-3'-methyl-3'-ethoxy)butyl-7-methoxy coumarin **8**. IR ν_{max}^{film} cm^{-1} 1725, 1624, 1565 and 825. UV λ_{max} nm 329, 298 (inf), 252, 242 (inf), 220 and 205 (log ε 3.86, 3.69, 3.64, 4.01 and 4.11). NMR ($CDCl_3$ 90 MHz) 2.41 (1H, *d*, *J* = 9.5 Hz; H-4), 2.81 (1H, *s*; H-5), 3.19 (1H, *s*; H-8), 3.75 (1H, *d*, *J* = 9.5 Hz; H-3), 6.05 (2H, *s*; CH_2—CO), 6.17 (3H, *s*; MeO), 6.54 (2H, *q*, *J* = 7 Hz; OCH_2Me), 8.64 (6H, *s*; *gem*-dimethyl) and 8.73 (3H, *t*, *J* = 7 Hz; OCH_2CH_3).

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REFERENCES

1. Estevez Reyes, R. and González, A. G. (1969) *Anales Quim.* **65(B)**, 91; Estevez Reyes, R. and Gonzàlez, A. G. (1971) *Anales Quim.* **67**, 207.
2. Estevez Reyes, R. and González, A. G. (1970) *Phytochemistry* **9**, 833.
3. González, A. G., Estevez Reyes, R. and Jaraiz, I. (1970) *Anales Quim.* **66** 1017; González, A. G., Estevez Reyes, R. and Jaraiz, I. (1972) *Anales Quim.* **68**, 425; González, A. G., Estevez Reyes, R. and Jaraiz, I. (1972) *Anales Quim.* **68**, 425; González, A. G., Estevez Reyes, R. and Jaraiz, I. (1971) *Phytochemistry* **10**, 1621; González, A. G., Estevez Reyes, R. and Jaraiz, I. (1970) *Phytochemistry* **9**, 1971.
4. Gonzàlez, A. G., Estevez Reyes, R., Bàez Arencibia, J. and Ruano Pérez, T. (1973) *Anales Quim.* **69**, 1141.
5. Estevez Reyes, R. and González, A. G. (1965) *Anales Fisica Quim.* **61(B)**, 803.
6. Estevez Reyes, R. and González, A. G. (1970) *Anales Quim.* **66**, 167.
7. King, F. E., Housley, J. R. and King, T. J. (1954) *J. Chem. Soc.* 1392.
8. González, A. G., Estevez Reyes, R. and Báez Arencibia, J. L. in preparation.

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PHLOROTANNINVORSTUFEN AUS *DICTYOTA DICHOTOMA**

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Key Word Index—*Dictyota dichotoma*; Dictyotales; brown algae; polyphenols; diphlorethol pentaacetate; difucol hexaacetate; identification.

Pflanze und Herkunft—*Dictyota dichotoma* (Hudson) *Lamouroux* (Roscoff/Bretagne, Sept. 1974, April 1975).

In Extrakten aus *D. dichotoma* wurde bisher nach der Acetylierung Phloroglucintriacetat (**1**) dc nachgewiesen [1]. Bei der Aufarbeitung größerer Mengen Algenmaterials wurden 4,5 mg **1** isoliert und durch MS, PMR-, IR-Spektren und Smp eindeutig identifiziert.

Neben **1** (R_f 0,59, Kieselgel-F_{254}-Fertigplatten Merck, $CHCl_3$–Me_2CO (47:3)) wurden zwei weitere UV-Licht löschende, mit Vanillin–H_2SO_4 rot färbbare Substanzen beobachtet: **2**, R_f 0,53 und **3**, R_f 0,49.

* Mitt. 21. 'Antibiotica aus Algen', Mitt. 20 s. Glombitza, K.-W., Wiedenfeld, G. und Eckhardt, G. *Arch. Pharm.* im Druck.