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STRUCTURE OF PINNARIN AND FUROPINNARIN, TWO NEW COUMARINS FROM THE ROOTS OF *RUTA PINNATA**

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Abstract—Seven coumarins were isolated from the roots of *Ruta pinnata* L. fil. Five were identified as xanthyletin, luvangetin, isopimpinellin, sabandin and thamnosin; the remaining two are new compounds, furopinnarin (I) and pinnarin (III). The tetrahydro derivative (II) was obtained from I and the dihydro derivative (IV) as well as isopinnarin (V), cyclopinnarin (VI), and limettin (VII) were obtained from III. Possible structures for sabandin (IX, X or XI) are discussed.

INTRODUCTION

Ruta pinnata L. fil. (Rutaceae) is endemic to the Canary Islands (Spain). In previous papers,[1-5] we reported the isolation of the following coumarins from leaves, twigs or fruits: coumarin, umbelliferone, herniarin, esculetin, scopoletin, psoralen, bergapten, xanthotoxin, isopimpinellin, oxypeucedanin, byakangelicin, marmesin, isobergapten, sphondin, luvangetin, seselin and sabandin, the last one being new in the literature. For sabandin, we then proposed two possible structures;[3] more recent experiments suggest that yet other structures are possible. Table 1 summarizes the distribution of the coumarins isolated from the aerial parts of the plant.

RESULTS AND DISCUSSION

This paper describes the investigation of the coumarins from the roots of *Ruta pinnata* which have already been the subject of a preliminary communication.[6] From the alcoholic extract we separated the essential oils by steam distillation; they consist of 94 per cent caryophyllene, 1 per cent methyl-*n*-pentyl ketone and traces of methyl-*n*-nonyl ketone, methyl-*n*-nonyl carbinol, coumarin, xanthyletin and pinnarin.[7] After washing the alcoholic extract

* Part XIV in the series "New Sources of Natural Coumarins".

[1] A. G. GONZALEZ and R. ESTEVEZ *An. Soc. Esp. Fis. Quim.* **B59**, 649 (1963).
[2] R. ESTEVEZ and A. G. GONZALEZ, *An. Soc. Esp. Fis. Quim.* **B59**, 765 (1963).
[3] R. ESTEVEZ and A. G. GONZALEZ, *An. Soc. Esp. Fis. Quim.* **B61**, 803 (1965).
[4] R. ESTEVEZ and A. G. GONZALEZ, *An. Soc. Esp. Fis. Quim.* **B62**, 981 (1966).
[5] R. ESTEVEZ and A. G. GONZALEZ, *An. Soc. Esp. Fis. Quim.* **B64**, 641 (1968).
[6] R. ESTEVEZ and A. G. GONZALEZ, *An. Soc. Esp. Fis. Quim.* **B65**, 91 (1969).
[7] R. ESTEVEZ and A. G. GONZALEZ, *An. Soc. Esp. Fis. Quim.* **B**, to be published.

TABLE 1. DISTRIBUTION OF COUMARINS IN *Ruta pinnata*

	Fruits[1,2]	Leaves[3,4]	Twigs[5]
Coumarins			
Coumarin	+	+	−
Umbelliferone	−	+	+
Herniarin	+	+	−
Esculetin	−	−	+
Scopoletin	−	−	+
Sabandin	−	++	−
Furocoumarins			
Psoralen	−	+	−
Bergapten	++	+	+
Xanthotoxin	+	+	−
Isopimpinellin	++	++	−
Oxypeucedanin	+	−	−
Byakangelicin	−	+	+
Marmesin	−	−	++
Isobergapten	+	−	−
Sphondin	++	−	−
Pyranocoumarins			
Luvangetin	−	−	++
Seselin	+	+	−

Key: ++ Found in major proportion; + found in small quantity; − absent.

with petroleum ether it was treated with benzene and the benzene extract was chromatographed on alumina. Eluting with petroleum ether/benzene and then with benzene and benzene/ethyl acetate we separated seven coumarins.

The first one eluted was xanthyletin, identified with authentic material through its physical constants and spectra (u.v., i.r., NMR, and MS).

The second coumarin eluted, *furopinnarin* (I), is a new compound. It has yellow u.v. fluorescence, empirical formula $C_{17}H_{16}O_4$ and m.p. 125°. Its u.v. spectrum (λ_{max} 254, 273, 316 nm) is similar to those of derivatives of psoralen with substituents at C_5 and C_8.[8-10] In the i.r., furopinnarin absorbs at ν_{max} 1723, 1593, 821 (coumarin), 911 (=CH_2), 1375, 1364 cm^{-1} (gem-dimethyl). Its NMR spectrum does not have any signals corresponding to benzene protons; it shows two doublets centred at τ 3·75 and 1·85 (2H; J=9·5 c/s) which may be associated with the coumarinic protons at C_3 and C_4, respectively; two doublets (2H; J=2.0 c/s) at τ 2·41 and 3·04 correspond to H_α and H_β of a furan; τ 5·83 (3H, s, OMe); τ 4·85 to 5·19 (2H, =CH_2); quadruplet at τ 3·33 to 3·83 (1H, —CH=); τ 8·23 (6H, s, gem-dimethyl). Its mass spectrum gives M^+ 284, the base peak at *m/e* 269 (M^+—Me) and a metastable peak at *m/e* 255; the prominent ions are in accord with the fragmentation expected for a psoralen derivative. The experimental results obtained for this furocoumarin are completely consistent with the proposed formula of 5-methoxy-8-(1′,1′-dimethylallyl)-psoralen (I). Concerning the isoprene side-chain, we believe it is at C_8 because the NMR signal for a proton at C_4 occurs at lower fields if C_5 bears a carbon–carbon bond, and also for biogenetic reasons.[11,12] On the other hand, the NMR signal for H-4 in furopinnarin and

[8] T. NAKABAYASHI, T. TOKORAYAMI, H. MIYASAKI and S. ISONO, *J. Pharm. Soc. Japan* **73**, 669 (1953); *Chem. Abs.* **47**, 10348 (1953).
[9] W. L. STANLEY and S. H. VANNIER, *J. Am. Chem. Soc.* **79**, 3488 (1957).
[10] W. L. STANLEY and S. H. VANNIER, *Phytochem.* **6**, 585 (1967).
[11] J. POLANSKI, *Bull. Soc. Chim. France*, 929 (1958).
[12] L. CROMBIE, D. E. GAMES and A. MCCORMICK, *Tetrahedron Letters* 151 (1966).

tetrahydrofuropinnarin agree with the data given in the literature for furocoumarins and their dihydro derivatives with a methoxyl group at C_5. This does not exclude the possibility that the isoprene chain might be attached at C_5.

Hydrogenating furopinnarin with Pd/C we obtained tetrahydrofuropinnarin (II), whose i.r. spectrum shows the characteristic bands of a coumarin (ν_{max} 1720, 1597, 826 cm^{-1}). In the u.v. it absorbs at λ_{max} 340 nm, which corresponds to λ_{max} 316 nm of a furocoumarin displaced by hydrogenation of the furanic to dihydrofuranic ring. The NMR signal of the methoxyl group is shifted to τ 6·06 (3H, s); the peak at τ 8·01 (2H, q) probably corresponds to the methylene group of the isoprene chain, its three methyl groups absorbing at τ 8·46 (6H, s) and 9·27 (3H, t). The prominent ions of the mass spectrum are in accordance with the expected fragmentation pattern.

Further elution yielded the second new coumarin, *pinnarin* (III), of empirical formula $C_{16}H_{18}O_4$ and m.p. 162–163°, showing blue u.v. fluorescence. In the i.r. it absorbs at 1713, 1595, 815 (coumarin), 1380–1387 (gem-dimethyl), 888 cm^{-1} (=CH_2). Its u.v. spectrum is similar to those of known coumarins with a methoxyl group at C_5 and C_7 and an isoprene side-chain at C_8 (see Table 2). The NMR spectrum of pinnarin shows doublets at τ 2·10 and

TABLE 2. ULTRAVIOLET ABSORPTION OF SOME 5,7,8-SUBSTITUTED COUMARINS (IN nm)

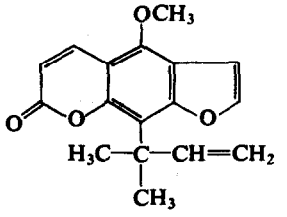
	λ_{max} (log ϵ)		
Pinnarin (III)	255 (sh, 3·97)	262 (3·99)	330 (4·17)
Mexotixin[13] or 5,7-dimethoxy-8-[CH_2—CH(OH)—C(OH)(CH_3)$_2$]-coumarin	252 (4·09)	260 (4·14)	326 (4·26)
5,7-Dimethoxy,8-(3′-methyl-2′-oxobutyl)-coumarin[14]	252 (3·86)	261 (3·91)	327 (4·06)
Coumurrayin[15] or 5,7-dimethoxy-8-[CH_2—CH=C(CH_3)$_2$]-coumarin	239 (3·77)	263 (3·99)	329 (4·09)
Sibiricin[16] or 5,7-dimethoxy-8-[CH_2—CH—C(CH_3)$_2$]-coumarin (O)	252 (sh, 3·92)	259 (sh, 3·98)	327 (4·19)

3·95 (J=10 c/s; 2H, coumarin β and α protons). The relatively low value for H_β suggests the presence of a methoxyl group at C_5 shielding the proton at C_4. Two singlets at τ 6·12 and 6·20 may be assigned to two methoxyl groups. Another singlet appears at τ 3·72 (1H), this same value being found in the literature for a benzene proton between two methoxyl groups. A series of peaks may be associated with a 1,1-dimethyl allyl group (τ 5·05 to 5·30, 2H, m, =CH_2; τ 3·55 to 4·00, 1H, m, —CH=; τ 8·35, 6H, s, Me_2C). The experimental results for pinnarin are consistent with the proposed structure of 5,7-dimethoxy-8-(1′,1′-dimethyl-allyl)-coumarin (III), confirmed by obtaining from it the corresponding dihydro derivative (IV), as well as isopinnarin (V), cyclopinnarin (VI), and limettin (VII).

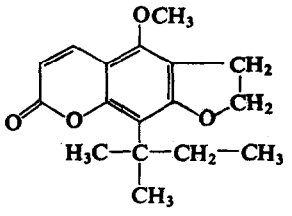
Hydrogenation of pinnarin with Pd/C gives dihydropinnarin (IV), $C_{16}H_{20}O_4$, m.p. 125°. Its i.r. spectrum shows the typical coumarin absorptions (ν_{max} 1710, 1590, 815 cm^{-1}), but lacks the one at 888 cm^{-1} which in pinnarin is assigned to the methylene group. The NMR spectrum is characterized by the following peaks: τ 8·43 (6H, s) corresponding to a gem-dimethyl group; τ 7·80 to 8·30 (2H, m) may be assigned to —CH_2—, and the triplet at τ 9·28

[13] D. P. CHAKRABORTY, B. K. CHOWDHURY and B. C. DAS, *Tetrahedron Letters* 3471 (1967).
[14] D. L. DREYER, *Tetrahedron* **23**, 4613 (1967).
[15] E. RAMSTAD, WEN-NUE C. LIN, TSUNG-JEN LIN and WEN-YAB KOO, *Tetrahedron Letters* 811 (1968).
[16] P. W. AUSTIN, T. R. SESHADRI, M. S. SOOD and VISHWAPAUL, *Tetrahedron* **24**, 3247 (1968).

(3H) to the methyl group in $—CH_2Me$; the other peaks are similar to those of pinnarin (see Experimental), indicating that the ring structure of the molecule has not been modified. In the mass spectrum appears a molecular ion at *m/e* 276 and prominent ions according to the expected fragmentation pattern. The results obtained for dihydropinnarin are consistent with the structure of 5,7-dimethoxy-8-(1′,1′-dimethyl-propyl)-coumarin (IV). Acid treatment of pinnarin yielded a mixture of products of which we isolated the three principal ones. Two of them, hitherto unknown, are called isopinnarin (V) and cyclopinnarin (VI) and the third was identified as limettin (VII) by direct comparison with an authentic sample (m.p., u.v., i.r., NMR, MS).



(I)



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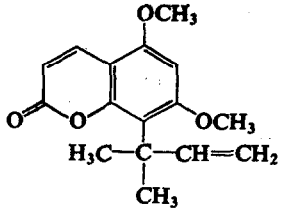

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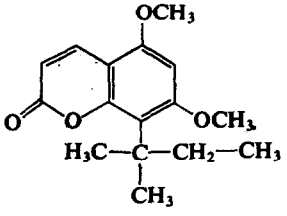
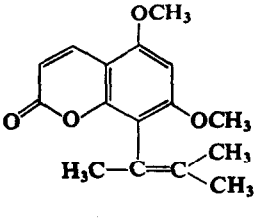
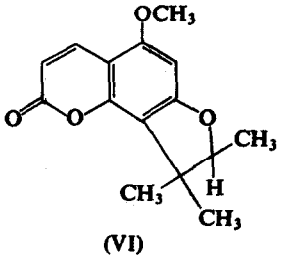


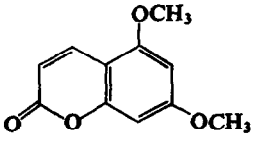
(IV)



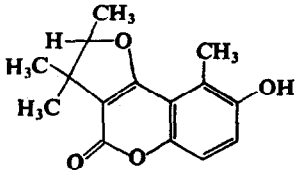
(V)



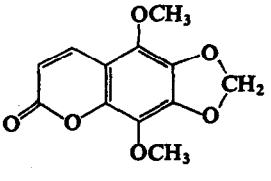
(VI)



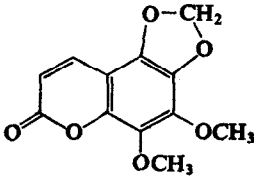
(VII)



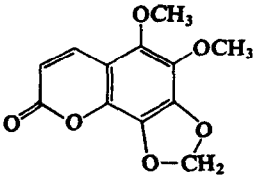
(VIII)



(IX)



(X)



(XI)

Isopinnarin (V), of empirical formula $C_{16}H_{18}O_4$ and m.p. 158–159°, showed yellow u.v. fluorescence. Its u.v. spectrum is similar to that of pinnarin (see Experimental). In the i.r. spectrum appear the coumarin absorptions (ν_{max} 1718, 1600, 803 cm^{-1}), the one at 888 cm^{-1}

being absent, which in pinnarin is assigned to the methylene group. The NMR data are consistent with structure V: τ 8·15 (6H, s) may be attributed to the group $=CMe_2$ and τ 8·57 (3H, s) to $=CMe$, the other peaks being similar to those of pinnarin. The mass spectrum shows a molecular ion at *m/e* 274 and prominent ions consistent with the fragmentation expected for structure V. The experimental results for isopinnarin coincide therefore with the structure of 5,7-dimethoxy-8-(1′-methyl-2′,2′-dimethylethenyl)-coumarin (V).

Cyclopinnarin (VI), of bluish violet u.v. fluorescence, absorbs in the u.v. at λ_{max} 345 nm (log ϵ 4·15), which corresponds to the values found in the literature for dihydrofurocoumarins; the remaining absorptions are similar to those of pinnarin (see Experimental). The i.r. spectrum shows bands at ν_{max} 1715, 1607 and 810 cm^{-1} (coumarin), the one at 888 cm^{-1}, which in pinnarin had been assigned to $=CH_2$, having disappeared. The NMR spectrum is coincident with structure VI: besides the peaks for the coumarin and aromatic protons it shows one at τ 5·50 (1H, q, J=6·5 c/s) which must correspond to —CH—O—; τ 8·62 (3H, d, J=6·5 c/s) may be assigned to the methyl group in —O—CH—Me and τ 8·48 and 8·75 (each 3H, s) to the gem-dimethyl. These values are consistent with those published by Irie *et al.*[17] for glaupalol (VIII), a coumarin with a trimethylated dihydrofuran ring identical to the one we think is present in cyclopinnarin. The mass spectrum is also in agreement with the proposed structure.

During the formation of cyclopinnarin by acid treatment of pinnarin, the methoxyl group at C_7 is demethylated, followed by cyclization; this is similar to the transformation of clausedin to cycloclausedin.[18] At the same time that pinnarin in acid medium undergoes these transformations, there must also occur a splitting-off of the isoprene chain to give limettin (VII).

After eluting pinnarin, the preparative chromatography yielded luvangetin, identified by comparison with authentic material (m.p., u.v., i.r., NMR, MS). Further elution gave isopimpinellin and sabandin, together representing 60 per cent of the coumarins isolated from the roots of *R. pinnata*. They may be separated either by direct chromatography on alumina with $AgNO_3$, or after hydrogenating the mixture, by chromatography on alumina which separates dihydroisopimpinellin from sabandin.

Isopimpinellin was identified through its m.p. and u.v., i.r., NMR and mass spectra, by comparison with an authentic sample. Hydrogenating isopimpinellin with Pd/C we obtained dihydroisopimpinellin, whose u.v., i.r. and NMR spectra are consistent with the structure of said dihydrofurocoumarin (see Experimental).

For sabandin we had provisionally proposed the structure of a furocoumarin,[3] but in view of some new experimental results we must revise it. Sabandin forms yellow needles of m.p. 145° with reddish yellow u.v. fluorescence, the empirical formula being $C_{12}H_{10}O_6$. In the i.r. it absorbs at ν_{max} 1710, 1615, 850 cm^{-1} (coumarin) and 937 cm^{-1} which may correspond to a methylenedioxy group. Its NMR spectrum shows peaks at τ 4·05 (2H, s) which may be attributed to a methylenedioxy group, and τ 6·05 and 6·10 (each 3H, s) assigned to two methoxyl groups. The mass spectrum has a molecular ion at *m/e* 250. The experimental results suggest any of the formulae IX, X or XI. In order to definitely establish the structure of sabandin, we are preparing it by synthesis.

Finally, the preparative chromatography yielded thamnosin. This coumarin was isolated for the first time from *Thamnosma montana* Torr. and Frem. by Dreyer *et al.*[19,20] Table 3

[17] H. Irie, K. Kinoshita, H. Mizutani, S. Uyeo and K. Yamamoto, *Yak. Zasshi* **88**, 627 (1968).
[18] B. S. Joshi, V. N. Kamat and A. K. Saksena, *Tetrahedron* **23**, 4785 (1967).
[19] D. L. Dreyer, *Tetrahedron* **22**, 2923 (1966).
[20] J. P. Kutney, T. Inaba and D. L. Dreyer, *J. Am. Chem. Soc.* **90**, 813 (1968).

summarizes the results obtained by gas–liquid and paper chromatography of the coumarins isolated from the roots of *R. pinnata* and of its derivatives.

TABLE 3. CHROMATOGRAPHIC DATA OF THE COUMARINS AND THEIR DERIVATIVES, FROM THE ROOTS OF *Ruta pinnata*

	GLC*	PC†	
		R_f in	
	T_r (min)	H_2O	H_2O/5% HOAc
Xanthyletin	17·6	0·35	0·50
Furopinnarin	31·5	0·0	0·0
Tetrahydrofuropinnarin	46·1	0·22	0·40
Pinnarin‡	27·3	0·0	—
Dihydropinnarin	29·5	0·17	0·32
Cyclopinnarin	17·8	0·34	0·51
Isopinnarin	24·4	0·54	0·68
Limettin	14·2	0·36	0·51
Luvangetin	33·0	0·43	0·49
Isopimpinellin	30·0	0·31	0·48
Dihydroisopimpinellin	87·8	0·50	0·60
Sabandin	36·5	0·51	0·61

* Column: QF-1; column temp. at the top: 174°; detector voltage: 1250 V; sensitivity: ×10; gas flow: 50 ml/min; chart speed: 6 in./hr.

† Ascending chromatography at 20° on Whatman No. 1 sheets (6–7 cm broad); total run: 30 cm.

‡ In PC, using the system butylene glycol/HOAc/H_2O (6 : 10 : 86) pinnarin has R_f 0·68.

EXPERIMENTAL

The m.p.'s, determined on a Kofler block, are uncorrected. The u.v. spectra were obtained in EtOH and the i.r. spectra in nujol. The NMR spectra were run in $CDCl_3$ with TMS as internal standard and TLC on silica gel Merck. Solvent used for recrystallizing coumarins was petrol. ether/benzene unless otherwise stated.

Extraction of Coumarins

The cortex (1.46 kg) of the roots of the wild form of *Ruta pinnata* L. fil. collected on the mountains near Bajamar (Tenerife) in November 1967 were extracted in a soxhlet with boiling EtOH. After eliminating the solvent by vacuum distillation, the extract was steam-distilled, thus separating the essential oils (36·6 g). The residue was dissolved in EtOH and mixed with deactivated alumina (800 g). This mixture was first extracted with petrol. ether (b.p. 50–80°), separating a viscous oil (72·0 g) whose investigation will be published later and afterwards with hot benzene yielding a dark-red product (83·3 g).

Chromatography of the Benzene Extract

After washing with petrol. ether, the benzene extract was mixed with deactivated neutral alumina (200 g), which was placed on the top of a column of alumina Merck (1·70 kg; activity II–III) in petrol. ether. Elution with petrol. ether gave small amounts of essential oils. Mixtures of petrol. ether–benzene separated substances which showed blue, yellow, light-blue and dark-blue u.v. fluorescence, respectively. With benzene alone we obtained one compound with greenish blue and two with yellow u.v. fluorescence. Finally, with benzene/AcOEt (1 : 1) a substance with brilliant light-blue fluorescence was eluted.

Xanthyletin

Blue u.v. fluorescence. In TLC, PC and GLC as well as by u.v. and i.r. analysis it behaved the same as an authentic sample of xanthyletin. M.p. 130°. Found: C, 73·56; H, 5·34. Calc. for $C_{14}H_{12}O_3$: C, 73·67; H, 5·30%. NMR: τ 2.50 (1H, d, J = 9.5 c/s), H-4), 3·03 (1H, s, H-5), 3·40 (1H, s, H-8), 3·50 (1H, d, J = 9·5 c/s, H-3), 3·73 (1H, d, J = 9.0 c/s, H-4'), 4·40 (1H, d, J = 9·0 c/s, H-3'), and 8·57 ppm (6H, s, Me_2). MS: M^+ 228 (MW calc. for $C_{14}H_{12}O_3$: 228); prominent ions m/e 213 (100%), 185 (32%); metastable peaks at m/e199 and 161.

Furopinnarin (I)

In PC, using the systems H_2O or 5% HOAc or butylene glycol/HOAc/H_2O (6:10:86) it did not move. M.p. 124–125° (from hexane/benzene). Found: C, 71·58; H, 5.71. $C_{17}H_{16}O_4$ required: C, 71·82; H, 5·67%. λ_{max} (log ϵ): 233 (4·12), 254 (3·96), 273 (3·97) and 316 nm (3·81). ν_{max} 1723, 1593, 1458, 1411, 1375, 1364, 1350, 1273–1265, 1215, 1183, 1156, 1143, 1105, 1000, 980, 911, 821, 730, 718 cm^{-1}, etc. NMR: τ 1·85 (1H, d, J = 9·5 c/s, H-4), 2·41 (1H, d, J = 2·0 c/s, H-2′), 3·04 (1H, d, J = 2·0 c/s, H-3′), 3·75 (1H, d, J = 9·5 c/s, H-3), 3·33–3·83 (1H, q, C*H*=CH_2), 4·85–5·19 (2H, m, CH=C*H*$_2$), 5·83 (3H, s, OMe), and 8·23 ppm (6H, s, gemdimethyl). MS: M^+ 284 (MW required for $C_{17}H_{16}O_4$: 284); prominent ions *m/e* 269 (100%), 257 (12%), 241 (17%), 229 (36%), etc.; metastable peak at *m/e* 255.

Tetrahydrofuropinnarin (II)

Obtained by hydrogenating furopinnarin in EtOH with 10% Pd/C. λ_{max} (log ϵ): 230 (4·33), 255 (3·98), 264 (3·97) and 340 mμ (4·32). ν_{max} 1720, 1597, 1550, 1410, 1385–1375, 1340, 1294, 1240, 1200, 1130, 1108, 1020, 990, 826, 800, 770, 720 cm^{-1}. NMR: τ 2·06 (1H, d, J = 10 c/s, H-4), 3·88 (1H, d, J = 10 c/s, H-3), 5·41 (2H, t, J = 6·5 c/s, H-2′), 6·06 (3H, s, OMe), 6·70 (2H, t, J = 6·5 c/s, H-3′), 8·01 (2H, q, C*H*$_2$Me), 8·46 (6H, s, gem-dimethyl), and 9·27 ppm (3H, t, CH_2*Me*). MS: M^+ 288 (MW required for $C_{17}H_{20}O_4$: 288); prominent ions *m/e* 259 (100%), 231 (35%), 217 (40%), 189 (60%).

Pinnarin (III)

Needles or quadrangular laminas, m.p. 162–163°. Found: C, 70·13; H, 6·58; OMe, 22·2. $C_{16}H_{18}O_4$ required: C, 70·07; H, 6·57; two OMe, 22·6%. U.v. spectrum: see Table 2. ν_{max} 1713, 1595, 1452, 1387–1380, 1260, 1230, 1217, 1170, 1125, 1100, 1000, 942, 888, 815, 770 cm^{-1}, etc. NMR: τ 2·10 (1H, d, J = 10 c/s, H-4), 3·55–4·00 (1H, m, C*H*=CH_2), 3·72 (1H, s, H-6), 3·95 (1H, d, J = 10 c/s, H-3), 5·05 and 5·30 (2H, m, CH=C*H*$_2$), 6·12 and 6·20 (each 3H, s, OMe), and 8·35 ppm (6H, s, gem-dimethyl).

Dihydropinnarin (IV)

Pinnarin (0.150 g) was hydrogenated with 10% Pd/C (0·070 g). Hydrogenation occurred rapidly. After filtering off the catalyst, the solution yielded a residue which, passed through alumina and recrystallized, had m.p. 125°. Found. C, 69·88; H, 7·42. $C_{16}H_{20}O_4$ required: C, 69·56; H, 7·24%. λ_{max} (log ϵ): 217 (4·45), 256 (sh, 3·99), 263 (4·01), and 330 nm (4·20). ν_{max} 1710, 1590, 1452, 1380, 1324, 1255, 1230, 1170, 1130, 1105, 1000, 947, 835, 815, 770 cm^{-1}, etc. NMR: τ 2·02 (1H, d, J = 10 c/s, H-4), 3·90 (1H, d, J = 10 c/s, H-3), 6·03 and 6·08 (each 3H, s, OMe), 7·80–8·30 (2H, m, C*H*$_2$Me), 8·43 (6H, s, gem-dimethyl), and 9·28 ppm (3H, t, CH_2*Me*). MS: M^+ 276 (MW required for $C_{16}H_{20}O_4$: 276); prominent ions *m/e* 247 (100%), 261 (9%), 233 (6%), 219 (80%), etc.; metastable peaks at *m/e* 247, 229, 221.

Acid Treatment of Pinnarin

Pinnarin (0·15 g), dissolved in glacial HOAc (4 ml), was gently heated on a water bath, whereupon conc. H_2SO_4 (2 drops) was added and the mixture left at room temp. for 1½ hr. It was then diluted with H_2O and extracted with ether. The solid obtained on evaporating the ether was chromatographed on a column of 45 g alumina + 10% $AgNO_3$ + 10% H_2O, mixtures of petrol.ether/benzene eluting cyclopinnarin (0·060 g), isopinnarin (0·025 g), limettin (0·024 g) and a mixture (0·030 g) of limettin with a second compound whose i.r. spectrum showed the presence of hydroxyl groups.

Isopinnarin (V)

Clustered needles, m.p. 158–159°. Found: C, 70·28; H, 6·84; OMe, 20·7. $C_{16}H_{18}O_4$ required: C, 70·06; H, 6·61; two OMe, 22·6%. λ_{max} (log ϵ): 225 (4·24), 255 (sh, 4·04), 263 (4·06), and 333 nm (4·18). ν_{max} 1718, 1600, 1500, 1465 (broad), 1380, 1332, 1285, 1243, 1215, 1190, 1160, 1125, 1105, 995, 938, 848, 820, 803 cm^{-1}, etc. NMR: τ 2·00 (1H, d, J = 10 c/s, H-4), 3·65 (1H, s, H-6), 3·86 (1H, d, J = 10 c/s, H-3), 6·07 and 6·12 (each 3H, s, OMe), 8·15 (6H) and 8·57 ppm (3H) both singlets assigned to —C(Me)=C(Me)$_2$. MS: M^+ 274 (100%; MW required for $C_{16}H_{18}O_4$: 274); prominent ions *m/e* 259 (45%), 244 (18%), 231 (75%), 216 (15%), etc.; metastable peaks at *m/e* 245, 230.

Cyclopinnarin (VI)

Blue-violet u.v. fluorescence which on treatment with alkali changes to blue-green. In TLC and on wetted paper it shows brilliant green u.v. fluorescence. In TLC, PC and GLC it behaved as a pure substance. Fine needles, m.p. 115–116° (from Pe). λ_{max} (log ϵ): 227 (4.17), 241 (sh, 3·86), 258 (sh, 3·96), 265 (4·01) and 345 nm (4·15). ν_{max} 1715, 1607, 1465, 1385, 1355, 1315, 1280, 1225, 1205, 1170, 1112, 1100, 1065, 1035, 993, 965, 870, 850, 830, 810 cm^{-1}, etc. NMR: τ 2·08 (1H, d, J = 10 c/s, H-4), 3·80 (1H, s, H-6), 3·95 (1H, d, J = 10 c/s, H-3), 5·50 (1H, q, J = 6·5 c/s, O—CH), 6·16 (3H, s, OMe), 8·62 (3H, d, J = 6·5 c/s, O—CH*Me*), 8·48 and 8·75 ppm (total 6H, s, gem-dimethyl). MS: M^+ 260 (MW required for $C_{15}H_{16}O_4$: 260); prominent ions *m/e* 245 (100%), 230 (14%), 217 (71%), 189 (32%), etc.; metastable peaks at *m/e* 231, 192, 164, etc.

Limettin (VII)

Blue-violet u.v. fluorescence. By chromatographic (TLC, PC, GLC) as well as u.v. and i.r. analyses it was found to be identical to an authentic sample of limettin. Needles, m.p. 144–145°. Found: C, 63·97; H, 4·97. Calc. for $C_{11}H_{10}O_4$: C, 64·08; H, 4·89%. NMR: τ 2·02 (1H, d, J = 10 c/s, H-4), 3·58 (1H, d, J = 2 c/s, H-6), 3·72 (1H, d, J = 2 c/s, H-8), 3·87 (1H, d, J = 10 c/s, H-3), 6·12 and 6·15 ppm (each 3H, s, OMe). MS: M^+ 206 (100%; MW calc. for $C_{11}H_{10}O_4$: 206); prominent ions *m/e* 178 (81%), 163 (48%).

Luvangetin

Blue-green u.v. fluorescence. In TLC, PC, GLC, u.v. and i.r. analysis it gave the same values as an authentic sample of luvangetin. Colourless needles, m.p. 109°. Found: C, 70·05; H, 5·41; OMe, 12·93. Calc. for $C_{15}H_{14}O_4$: C, 69·72; H, 5·43; one OMe, 12·02%. NMR: τ 2·46 (1H, d, J = 9·5 c/s, H-4), 3·20 (1H, s, H-5), 3·68 (1H, d, J = 9 c/s, H-4'), 3·83 (1H, d, J = 9·5 c/s, H-3), 4·34 (1H, d, J = 9 c/s, H-3'), 6·05 (3H, s, OMe), and 8·50 ppm (6H, s, gem-dimethyl). MS: M^+ 258 (MW calc. for $C_{15}H_{14}O_4$: 258); prominent ions *m/e* 243 (100%), 228 (44%), 215 (17%), 200 (42%); metastable peaks at *m/e* 229, 214, 175.

Isopimpinellin

GLC revealed that isopimpinellin and sabandin represent 60% of the coumarins in the benzene extract of the roots of *R. pinnata*. By TLC and PC they are both easily separated from one another, but on preparative scale the separation is more difficult. The problem was resolved by chromatographing the mixture on acid alumina and on alumina with $AgNO_3$. Isopimpinellin, showing reddish u.v. fluorescence, crystallized as yellow needles, m.p. 149–150°. Its chromatographic (TLC, PC, GLC) and spectral (u.v., i.r., NMR, MS) data were the same as those of an authentic sample.

Dihydroisopimpinellin

The mixture of isopimpinellin and sabandin (0·51 g) in EtOH (60 ml) was hydrogenated with 10% Pd/C and then chromatographed on neutral alumina Merck (40 g; activity II–III). Elution with benzene yielded sabandin and dihydroisopimpinellin separately. Dihydroisopimpinellin, brilliant yellow u.v. fluorescence, m.p. 143–144°. λ_{max} (log ϵ): 230 (4·44), 258 (sh, 3·84), 265 (3·91) and 338 nm (4·20). ν_{max} 1725, 1610, 1582, 1410, 1376, 1280, 1207, 1130, 1098, 1065, 1010, 970, 830 cm^{-1}, etc. NMR: τ 2·13 (1H, d, J = 10 c/s, H-4), 3·90 (1H, d, J = 10 c/s, H-3), 5·32 (2H, t, J = 6·5 c/s, H-2'), 6·08 (6H, s, 2 OMe), and 6·61 ppm (2H, t, 6·5 c/s, H-3').

Sabandin (IX, X or XI)

Yellow needles, m.p. 145°. Found: C, 57·70; H, 4·16; OMe 24·31. $C_{12}H_{10}O_6$ required: C, 57·61; H, 4·03; two OMe, 24·72%. λ_{max} (log ϵ): 228 (4·36), 242 (sh, 4·30), and 328 nm (4·26). ν_{max} 3078, 1710, 1615, 1590, 1480, 1420, 1376, 1280, 1150, 1090, 1070, 993, 975, 952, 937, 875, 850, 806 cm^{-1}, etc. NMR: τ 2·12 (1H, d, J = 9·5 c/s, H-4), 3·87 (1H, d, J = 9·5 c/s, H-3), 4·05 (2H, s, O—CH_2—O), 6·05 (3H, s, OMe), and 6·10 ppm (3H, s, OMe). MS: M^+ 250 (MW required for $C_{12}H_{10}O_6$: 250); prominent ions *m/e* 235 (100%), 222 (8%), 207 (23%), 177 (23%); metastable peak at *m/e* 221.

Thamnosin

Brilliant blue u.v. fluorescence. By TLC in several systems it behaved as a pure substance. Quadrangular plates, m.p. 240° (from petrol.ether/$CHCl_3$). Its u.v. and i.r. data proved to be the same as those published by Dreyer *et al.*[19,20] NMR: τ 2·45 and 2·50 (each 1H, d, J = 10 c/s; 2 H-4), 2·90 and 2·95 (each 1H, s; 2 H-5), 3·38 (2H, s, 2 H-8), 3·85–3·92 (4H, m, 2 H-3 and *H*C=C*H*), 4·75 (1H, m, *H*C—CMe), 6·20 (1H, Ph—C*H*—Me), 6·28 (6H, s, 2 MeO), 7·85 [2H, m, J = 7 c/s, C=C(Me)—CH_2—CH_2—], 8·20 (3H, s, C=CMe), 8·35 [2H, m, J = 7 c/s, C=C(Me)—CH_2—CH_2—] and 8·75 ppm (3H, s, Ph—C*Me*). MS: M^+ 484 (MW calc. for $C_{30}H_{28}O_6$: 484); prominent ions *m/e* 242 (100%), 211 (55%), 183 (16%); metastable peak at *m/e* 213.

Dihydrothamnosin

This derivative was prepared by hydrogenating thamnosin (0·28 g) with 10% Pd/C in EtOH/$CHCl_3$. The u.v. and i.r. spectra were consistent with those determined by Dreyer *et al.*[19,20] NMR: τ 2·43 and 2·50 (each 1H, d, J = 9 c/s; 2 H-4), 2·90–3·30 (4H, 4 peaks, 2 H-5 and 2 H-8), 3·82 (2H, d, J = 9 c/s, 2 H-3), 4·81 (1H, m, *H*C=CMe), 6·15–6·20 (7H, 2 OMe and Ph—*H*C=CMe), 7·54 (2H, q, J = 7 c/s, Ph—CH_2), 7·90 [2H, m not resolved, C=C(Me)—CH_2], 8·24 [3H, s with broad base, C=C(*Me*)—CH_2], 8·33 [2H, m not resolved, C=C(Me)—CH_2—CH_2], 8·63 (2H, q, J = 7 c/s, Ph—CH_2—CH_2—) and 8·93 ppm (3H, s, Ph—CH—C*Me*).

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