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STRUCTURE OF SABANDININ AND OTHER COUMARINS ISOLATED FROM THE ROOTS OF *RUTA PINNATA**

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Abstract-Gravelliferone methyl ether (I), xanthyletin, furopinnarin (IV), pinnarin (VI), benahorin (VII), limettin, luvangetin, bergapten (V) and a new coumarin sabandinin (X) were isolated from the roots of *Ruta pinnata* L. fil. The structures of I, IV and VII were confirmed by degradative studies.

INTRODUCTION

Ruta pinnata L. fil. (Rutaceae), endemic to the Canary Islands, is an excellent source of coumarins. [1-3] Previously,[3] we examined the benzene-soluble fraction of the alcoholic extract of the roots and succeeded in isolating xanthyletin, luvangetin, thamnosin, isopimpinellin, pinnarin (IV), furopinnarin (VI) and sabandin, the last three compounds being new. Of the three possible structures then suggested for sabandin,[3] formula XI best agreed with the spectroscopic data.[4]

The present communication reports the coumarins isolated from the petroleum-soluble fraction of the steam-distilled alcoholic extract of the roots, which by column chromatography on alumina yielded gravelliferone methyl ether (I), xanthyletin, furopinnarin (IV), pinnarin (VI), limettin, luvangetin, benahorin (VII),[4] bergapten (V) and finally a new coumarin which, due to its close relationship to sabandin, we have called sabandinin (X).

RESULTS AND DISCUSSION

The first coumarin eluted, gravelliferone methyl ether (I) (NMR: Table 1), was recently isolated from *Ruta graveolens* L. by Reisch *et al.*[5] who proposed structure I based on elementary and spectroscopic analyses. We found that acid treatment of I gave a mixture of four coumarins which were separated by chromatography on alumina. NMR showed three of them to be isomers of I. The fourth one was identified as 3-(1′,1′-dimethylallyl)-herniarin (II), also isolated by Reisch et *al.*[5] from *Ruta graveolens* L., its NMR spectrum

* Part XVII in the series "New Sources of Natural Coumarins". for Part XVI, see R. ESTÉVEZ and A. G. GONZÁLEZ, *Anal. Quim.* (in press).

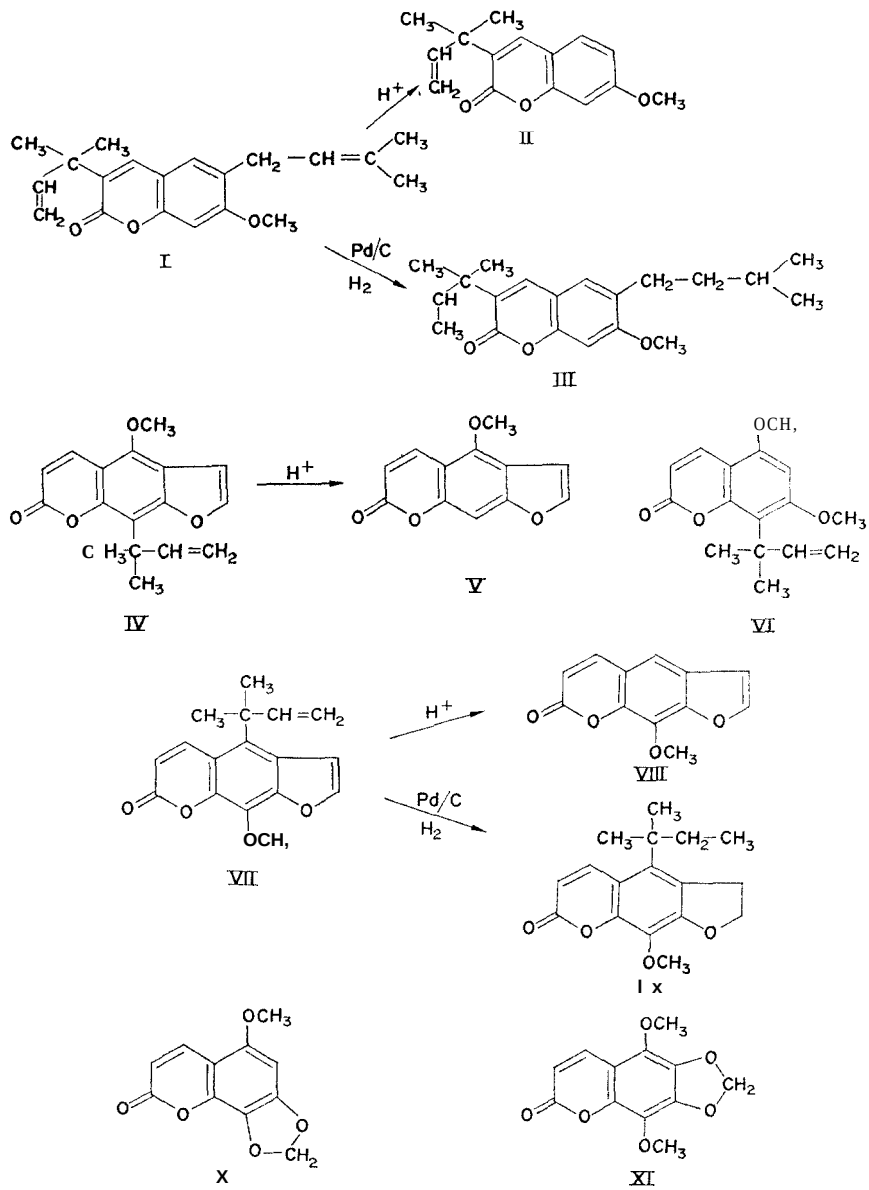
[1] A. G. GONZÁLEZ and R. ESTÉVEZ, *Anal.* Fis. Quim. **59B,** 649 (1963); R. ESTÉVEZ GONZÁLEZ, *Anal. Fis.* Quim. **59B,** 765 (1963); **61B,** 803 (1965); **62B,** 981 (1966); **64B,** 641 (1968); **65B,** 91 (1969).

[2] R. ESTÉVEZ and A. G. GONZÁLEZ, *Anal. Quim. 66, 167 (1970).*

[3] R. ESTÉVEZ and A. G. GONZÁLEZ, *Phytochem.* 9,833 (1970).

[4] A. GONZÁLEZ GONZÁLEZ, E. AGULLÓ MARTÍNEZ, MA A. MARTÍNEZ IÑIGUEZ and F. RODRÍGUEZ LUIS, *Anal. Quim.* (in press).

[5] J. REISCH, K. SZENDREI, E. MINKER and I. NOVAK, *Tetrahedron Letters 4395 (1968).*



(Table 1) being in accord with formula II. It thus represents a contribution to the structure of gravelliferone methyl ether. Hydrogenation of I over Pd/C yielded tetrahydrogravelliferone methyl ether (III) (NMR: Table 1).

The next coumarins isolated, xanthyletin, furopinnarin (IV), pinnarin (VI), limettin and luvangetin, were identified by direct comparison with authentic samples. In the mass spectrum of pinnarin (see Experimental) the intensity of the molecular peak (*m/e* 274) is 78 %, indicating the great stability of this coumarin. Acid treatment of furopinnarin gave bergapten (V), which thus confirms the proposed structure[3] of 5-methoxy-8-(1′,1′-dimethylallyl)-psoralen (IV).

TABLE 1. τ-VALUES IN $CDCl_3$ (IN PARENTHESES: I-VALUES)

Compound	H-3	H-4	H-5	H-6	H-8	H-2	H-3′	OCH_2O	CH_2=CH=	=CH	$C(Me)_2\phi$—$CH_2\phi$—CH_2—CH=$C(Me)_2$	=C(Me)$_2$	—CH OMe
Gravelliferone methyl ether (I)		2·50 s	2.86 s		3.28 s				3·58–4·04 q	4.85 m 5·08 m	5·54 s	4·63 t (7) 6·71 d (7)	8.27 d (4)	6.16 s
3-(1′,1′-Dimethyl-allyl)-herniarin (II)		2.48 s	2.70 d (9)	3.15 d (9)	3·14 s				3·56–4·01 q	4·90 m 5·13 m	8.57 s			6.20 s
Tetrahydrogravelliferone methyl ether (III)		2·60 s	2.83 s		3·29 s				8.15 q* (8)	9·30 t† (8)	8·70 s	8.54 m‡ (8) 7·41 m (7)	9·06 d§ (6)	7·2–7·7 m 6.17 s
Furopinnarin (IV)[3]	3.75 d (10)	1.85 d (10)				2.41 d (2)	3·04 d (2)		3·33–3·83 q	4.85 m 5.19 m	8.23 s			5·83 s
Bergapten (V)	3·76 d (10)	1·85 d (10)			2·90 s	2.39 d (2)	2.97 d (2)							5.74 s
Pinnarin (VI)[3]	3.95 d (10)	2·10 d (10)		3.72 s					3·55–4·00 q	5·05 m 5·30 m	8.35 s			6.12 s 6.20 s
Benahorin (VII)	3.77 d (10)	1.54 d (10)				2·36 d (2)	2.75 d (2)		3·46–3.92 q	481 m 5·19 m	8·29 s			5·80 s
Xanthotoxin (VIII)	3·66 d (10)	2·24 d (10)	2.35 s			2.35 d (2)	3·20 d (2)							5.73 s
Tetrahydrobenahorin (IX)	3.83 d (10)	1.65 d (10)				5·40 t (7)	6·46 t (7)		8·10 q* (8)	9.23 t† (8)	8·49 s			6.20 s
Sabandinin (X)	3.82 d (10)	2·06 d (10)		3.52 s				4·06 s						5·91 s

* CH_2—CH_3.
† CH_2—CH_3.
‡ ϕ—CH_2—CH_2—.
§ —$CH(Me)_2$.

Between pinnarin and limettin we eluted an oily substance with a yellow-ochre u.v. fluoresence which crystallized from EtOH–H_2O as large quadrangular plates of m.p. 88-90" identified as benahorin (VII) (NMR: Table 1). For this compound, recently isolated from the twigs of *Ruta pinnata* L. fil., we proposed the structure 5-(1′,1′-dimethylallyl)-8-methoxypsoralen (VII) based on spectroscopic studies.[4] Acid treatment of this furocoumarin under stronger conditions than those used for the degradation of gravelliferone methyl ether and furopinnarin, gave xanthotoxin (VIII) in 20% yield (NMR: Table 1). This confirms the structure suggested for benahorin, an isomer of furopinnarin. In the mass spectrum of VII the base peak is given by the molecular ion, which proves that the molecule is very stable. Hydrogenation with Pd/C gave tetrahydrobenahorin (IX) which has u.v. absorption at 343 nm characteristic of dihydrofurocoumarins. In its NMR spectrum (Table 1) the doublet attributed to the H-4 appears at τ 1·65, slightly displaced with respect to the corresponding one in benahorin (τ 1.54) due to the deshielding caused by hydrogenating the furan ring.

Finally, light petroleum-benzene (1: 5) eluted a mixture of two coumarins, one of them being identified by TLC as bergapten (V) (NMR: Table 1). As their separation proved to be difficult, the mixture was first hydrogenated with Pd/C and then chromatographed on alumina, separating dihydrobergapten from the new coumarin sabandinin (X) which had not undergone any alteration. It has the empirical formula $C_{11}H_8O_5$, m.p. 193–194°, very soft greenish yellow u.v. fluorescence and shows the typical u.v. absorption of a simple coumarin. The chemical shifts (Table 1) are in agreement with the structure of 5-methoxy-7,-8-methylenedioxycoumarin (X), the singlet at τ 3·52 being characteristic of the H-6 in a coumarin with substituents OR on the adjacent C atoms. The NMR signals τ 2·15 for H-4 and 3·05 for H-5, present in the isomer 6-methoxy-7,8-methylenedioxycoumarin recently isolated by Herz *et al.*[6] from *Artemisia dracunculoides* Pursh, confirm our assignations for H-4 and H-6 in sabandinin.

From the mother liquors of limettin and luvangetin, we obtained a compound of blue u.v. fluorescence and m.p. 130–132°, the i.r. spectrum of which is that of a simple coumarin with free hydroxyl groups (ν_{max} 3520, 1695, 1572, 826 cm-'). Its u.v. absorptions indicate that C_6 probably bears a substituent and C_7 an OR group (see Experimental). Its complete structure is now under investigation,

EXPERIMENTAL

The m.ps, determined on a Kofler block, are uncorrected. Solvent used for recrystallizing coumarins was light petroleum (50–80°)–benzene unless otherwise stated. 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. Column chromatography was carried out on alumina Merck, activity II-III, TLC on silica gel Merck, and PC on Whatman No. 1, ascending technique.

Extraction and Chromatography of Coumarins

The cortex (1.46 kg) of the roots of *Ruta pinnata* L. fil. was extracted with boiling EtOH and the extract steam-distilled. The composition of the essential oils thus obtained is published elsewhere.* The remaining residue was first extracted with light petroleum and then with benzene, the results of the latter being published already.[4] The viscous oil (72·0 g) obtained from the extract with light petroleum was chromatographed on alumina (1·40 kg) eluting withlight petroleum, mixturesof light petroleum-benzene,andbenzene.

Gravelliferone Methyl Ether (I)

Eluted with light petroleum, blue u.v. fluorescence, m.p. 70" (from EtOH–H_2O). Found: C, 76.98; H, 7.75. Calc. for $C_{20}H_{24}O_3$ (mol. wt. 312); C, 76.92; H, 7.75%. MS: M+ 312 (95%); prominent ions m/e 297 (100%, M+-Me), 285 (8%, M+ $-CH{=}CH_2$), 269 (26%, M^+ — CO, — Me). λ_{max} (log ϵ): 223 (4·31), 245 sh (3·94), 255 (3·78), 285 sh (3·89), 298 sh (4·05), 332 (4.36); λ_{min} 264 nm(3.14). ν_{max} 1720, 1620, 1573, 1268, 1196, 1122, 1025, 988,905, 878, 820, 780 cm-r. NMR: Table 1.

[6] W. HERZ, S. V. BHAT and P. S. SANTHANAM, *Phytochem. 9,891 (1970).*

Acid Treatment of I: 3-(1′,1′-Dimethylallyl)-herniarin (IZ)

Gravelliferone methyl ether (0·45 g) dissolved in HOAc (5 ml) was treated with conc. H_2SO_4 (3 drops) for 1 hr at 100". The solution was cooled, diluted with H_2O and extracted with ether. After washing the organic layer with H_2O and aq. $NaHCO_3$, the solvent was evaporated and the residue chromatographed, yielding four compounds, one of them identified as 3-(1′,1′-dimethylallyl)-herniarin (II) showing blue u.v. fluorescence. NMR: Table 1.

Tetrahydrogravelliferone Methyl Ether (ZZZ)

Hydrogenation of I with 10% Pd/C at room temp. under atm. press. gave the tetrahydro derivative (III) as viscous oil of blue u.v. fluorescence which did not crystallize. λ_{max} (log ϵ): 225 (4·52), 244 sh (4·02), 255 sh (3·82), 284 sh (3·98), 298 sh (4·40), 334 (446); λ_{min} 268 nm (3.28). ν_{max} 1710, 1617, 1582, 1275, 1255, 1200, 1140, 1020, 986, 930, 830, 787 cm^{-1}. NMR: Table 1:

Xanthyletin, Furopinnarin (IV), Pinnarin (VI), Limettin and Luvangetin

They were identified by direct comparison with authentic samples (mixed m.p.,* TLC, PC, u.v., i.r., NMR). MS of pinnarin (VI): M^+ 274 (78%; mol. wt. required for $C_{16}H_{18}O_4$: 274); prominent ions m/e 259 (100%, M+-Me), 247 (4%, M^+—$CH{=}CH_2$), 231 (33%, M+-CO, -Me), 219 (28%, M+-CO, -CH=CH_2), 205 (6%, M^+—$C(Me)_2CH{=}CH_2$). NMR of IV and VI: Table 1.

Acid Treatment of Furopinnarin (IV): Bergapten (V)

IV (102 mg) was treated as mentioned above for I. Chromatography yielded bergapten (50 mg) identified by direct comparison with authentic material (mixed m.p., TLC, PC; u.v. and i.r. spectra superposable). NMR: Table 1.

Benahorin (VII)

Oily substance eluted with light petroleum-benzene (1: 2) between the fractions which contained pinnarin and those with limettin. Sparingly soluble in light petroleum, it crystallized with difficulty, after several days, from EtOH containing traces of H_2O, m.p. 88–90°, and was identified by comparing it with an authentic sample.' *Found: C, 71.73;* H, 5.57. Calc. for $C_{17}H_{16}O_4$ (mol. wt. 284): C, 71·82; H, 5.67%. MS: M^+ 284 (100%); prominent ions m/e 269 (38%, M^+ — Me), 257 (7%, M^+ — $CH{=}CH_2$), 241 (21%, M^+ — Me, — CO). I,, (log ϵ): 226 (4·32), 251 (4·27), 264 (4·25), 313 (409); λ_{min} 236 (4·28), 261 (4·24), 280 nm (3·63). ν_{max} 1720, 1580, 1278, 1168, 1104, 1058, 1002, 834, 765 cm^{-1}. NMR: Table 1.

Acid Treatment of VII: Xanthotoxin (VIII)

Benahorin (0·22 g) was treated as described above for I, but heating the mixture for 2 hr at 100° and monitoring the reaction by TLC. Chromatography of the residue yielded, besides starting material, xanthotoxin (50 mg) identified by direct comparison with an authentic sample (mixed m.p., TLC, PC; u.v. and i.r. spectra superposable). Greenish yellow u.v. fluorescence, m.p. 146-147". NMR: Table 1.

Tetrahydrobenahorin (IX)

VII (0.25 g) dissolved in EtOH (50 ml) was hydrogenated with 10% Pd/C (0.20 g) at room temp. under atm. press. yielding the tetrahydro derivative (IX), of strong pale blue u.v. fluorescence and m.p. 150–151° (from EtOH). Found: C, 70·72, H, 7.08. $C_{17}H_{20}O_4$ required: C, 70.81: H, 690%. λ_{max} (log ϵ): 230 (4·54), 254 (4·06), 264 (4·07), 333 sh (4·46), 343 (4.48); λ_{min} 249 (4·05), 260 (4·06), 278 nm (3.46). ν_{max} 1715, 1567, 1345, 1278, 1242, 1142, 1106, 1082, 1020,993, 978, 830, 765 cm^{-1}. NMR: Table 1.

Separation of Sabandinin (X) from Bergapten (V)

Light petroleum-benzene (1: 5) eluted a crystalline product which by TLC was shown to consist of two compounds of greenish yellow u.v. fluorescence, one of them identified as bergapten (TLC, PC). As they could not be separated by column chromatography, the mixture (70 mg) was hydrogenated in EtOH over 10% Pd/C at room temp. and atm. press. On chromatography of the resulting product, light petroleum–benzene (1: 2) eluted sabandinin (X), the second compound of the original mixture, and benzene eluted dihydrobergapten (with hydrogenated furan ring). The latter had intense blue u.v. fluorescence and m.p. 165–166" and was compared with an authentic sample (mixed m.p., TLC, PC; u.v. and i.r. spectra superposable).

Sabandinin (X)

M.p. 193-194" (ivory plates). Found: C, 60·26; H, 3·92. $C_{11}H_8O_5$ required: C, 60·00; H, 364%. λ_{max} (log ϵ): 202 (4·70), 212 sh (4·50), 238 (4·04), 257 sh (3·67), 317 (4·03), 330 sh (4.00) λ_{min} 230 (4·02), 270 nm (3·61). ν_{max} 1725, 1622, 1580, 1395, 1300, 1247, 1210, 1120, 1098, 1045, 942, 878, 830, 810 cm^{-1}. NMR: Table 1.

* Recrystallized from benzene, furopinnarin (m.p. 128-129") and pinnarin (m.p. 173-174") give somewhat higher m.ps than those published earlier (124-125" and 162–163°, respectively).[3]

Hydroxycoumarin, m.p. 130–132°

From the mother liquors of limettin and luvangetin was obtained a colorless compound (8 mg) of m.p. 130-132° (from benzene) and blue u.v. fluorescence which by TLC and PC was shown to be pure. λ_{max} *221, 242* sh, 252, *296* sh, 327; λ_{min} *262* nm. υ_{max} 3520, 1695, 1618, 1572, 1270, 1194, 1138, 1008, 990, 915, 826, *780* cm^{-1}.

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