Distribution of compounds in fractions of propolis extract

Compound	Fractions NaOH*	Na_2CO_3**	HCl**
Pinostrobin (**1**)	4%	+	+
Sakuranetin (**2**)	3%	-	+
Isosakuranetin (**3**)	-	+	-
Flavanone (**4**)	-	+	+
Pterostilbene (**5**)	10%	-	+
Xanthorrhoeol (**6**)	2%	+	-
3,5-Dimethoxybenzyl alcohol (**7**)	-	-	+

*Amount of each compound shown as percentage of the material in that fraction. **+ sign indicates presence of a compound in small amounts.

preparative TLC techniques were used in the usual way. The compounds isolated and identified in the different fractions examined are listed in the table. Evidence for the structure of the 4 flavanones isolated was obtained from an interpretation of their spectral characteristics following well-established approaches[10,11] and by comparison of the physical properties with those described in the literature. The flavanones identified were: (S)-(−)-Pinostrobin (**1**), m.p. 110–111 °C, $[\alpha]_D$ −54° (Lindstedt[12], 112–113 °C, $[\alpha]_D$ −56°), NMR (60 MHz, CCl_4), δ 2.5–3.4 (AB multiplet of an ABX system, 3-H_2), 3.80 (aromatic methoxyl), 5.36 (X part, $|J_{AX} + J_{BX}|$ 16 Hz, 2-H), 5.94 (6- and 8-H), 7.36 (5 aromatic protons), 11.92 (OH), MS (m/e, %) 270 (M^+, 100), 269 (50), 193 (90), 166 (65), 138 (45), 104 (25); (S)-(−)-Sakuranetin (**2**), m.p. 152–154 °C, $[\alpha]_D$ −6° (Dean[13] 150 °C, $[\alpha]_D$ −10°), NMR (90 MHz, $CDCl_3$) 2.75 and 3.10 (AB part of an ABX system, J_{AB} 17 Hz, J_{AX} 4.5 Hz, J_{BX} 11 Hz, 3-H_2), 3.80 (aromatic methoxyl), 5.30 (X part, $|J_{AX} + J_{BX}|$ 15.5 Hz), 5.99 and 6.04 (AB system, J_{AB} 2.5 Hz, 6- and 8-H), 6.85 and 7.31 (AA'BB' system, B-ring protons), 11.99 (5-OH), MS (m/e, %) 286 (M^+, 100), 285 (42), 193 (33), 166 (100), 138 (25), 120 (92); Isosakuranetin (**3**) (NMR and MS identical with those of an authentic sample) and (−)-5-hydroxy-4',7-dimethoxyflavanone (**4**), m.p. 114.5–115 °C, $[\alpha]_D$ −26° (Bohm[14], m.p. 116.5–117 °C), NMR and MS identical with those reported[14]

A major constituent of the NaOH soluble fraction was identified as pterostilbene (**5**) (m.p. 80–81 °C; Spath et al.[15], 85–86 °C); NMR (90 MHz; $CDCl_3$), δ 3.81 (aromatic methoxyl), 6.37 (triplet, J 2.5 Hz, 4-H), 6.63 (doublet, J 2.5 Hz, 2- and 6-H), 6.87 and 7.00 (AB quartet, J 16 Hz, vinylic protons), 6.81 and 7.36 (AA'BB' system); IR ($CHCl_3$), ν 3610, 2850 cm^{-1}; UV, λ_{max} 217 nm (ε 20,000), 315 nm (ε 22,500); MS (m/e, %) 256 (M^+, 100), 240 (6), 225 (7) 210 (6), 197 (6), 181 (15), 169 (6), 152 (9), 128 (10), 115 (10). The next compound isolated was shown to be 4-acetyl-5-hydroxy-2-methyl-2*H*-3*H*-naphtho (1,8-*b*,*c*) pyran (xanthorrhoeol, **6**) (m.p. 120–121°[undepressed on admixture with an authentic sample], $[\alpha]_D$ + 136°; Duewell[16], m.p. 121 °C, $[\alpha]_D$ + 143°, NMR-, IR-, UV- and mass spectra identical to those reported[16,17]). The presence in the acid-soluble fraction of 3,5-dimethoxybenzyl alcohol (**7**) was confirmed by comparison of its spectral data and GC retention time with those of an authentic sample. As shown in the table this fraction also contained amounts of **1**, **2**, **4** and **5**. A possible explanation is that these compounds were initially present, in part, as water soluble glycosides which were then hydrolyzed on contact with aqueous acid.

Given these results a number of points are worth mentioning with reference to the origin and the possible pharmacological effects attributed to propolis.

Xanthorrhoeol (**6**), sakuranetin (**2**), isosakuranetin (**3**) and **4** have previously been found[16,17] in *Xanthorrhoea* species, the 'grass trees' endemic to Australia. Although polyhydroxy- and polymethoxystilbenes are commonly found[18] in *Eucalyptus* species, pterostilbene (**5**) has not yet been reported as a constituent. The substitution pattern found in the benzyl alcohol (**7**) indicates that it may be a degradation product of pterostilbene.

The pharmacological activity of xanthorrhoeol and pterostilbene is not known, although the latter has been reported to be useful in the treatment of diabetes[19] and, in common with other stilbenes, may show antifungal activity[20]. It has been claimed that sakuranetin shows fungicidal activity[21,22] and isosakuranetin antinephrotoxic properties[23].

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Hypotensive activity of some dihydroxycoumarins and their congeners

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Summary. Evaluation of the hypotensive activity of dihydroxy coumarins and their congeners reveal that the naturally occurring dimethoxy coumarin Scoparone has maximal activity, more significant than L-α-methyl dopa. Structure activity relationship studies are reported with an attempt to offer a probable mechanism of action.

Scoparone (6,7-dimethoxycoumarin, **I**) occurring in *Artemisia scoparia* Waldst. & Kit. as the major constituent, has shown marked hypotensive and tranquillising activity[1,2]. This natural prototype may not be the best representative exhibiting this physiological response; hence the present study was undertaken to evaluate minimum structural requirements for the hypotensive activity against the congeners of the naturally occurring coumarin profile.

It has been reported that L-α-methyl dopa (**II**), a potent antihypertensive agent, possesses a structural framework which manifests the specific biological response which prompted Rastogi and his coworkers[3] to synthesise various 3-amino-3-methyl-6, 7dihydroxy 3-4-dihydro coumarins and substituted tetralins, and Taylor et al.[4] to prepare indan derivatives. We now report that **I** exhibits better hypotensive activity than **II** and also provides the rigidity of molecular framework due to lactone ring.

Material and method. Aesculetin (**XII**), 5-7-dihydroxy coumarin (**X**), 7-hydroxy coumarin (**IV**) were synthesised from the appropriate phenols and malic acid in the presence of acid catalyst according to Pechmann condensation[5]. These parent hydroxy coumarins were alkylated and esterified according to general method[6]. Knoevengal reaction[7] afforded the 4-methyl coumarins, **IX**, **XIV**, **XVII** in which case ethyl acetoacetate was condensed with the corresponding phenols. **XXI** was obtained from **V** through Elb's persulphate oxidation[8].

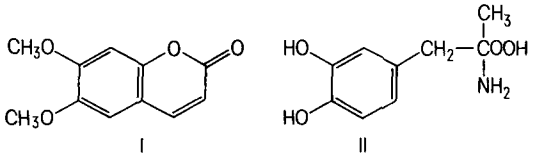
For pharmacological experiments mongrel dogs of either sex (4–7 kg) anaesthetised with quinalbarbitone sodium (30 mg/kg) were used. Right common cartoid artery was cannulated and connected to the mercury manometer for recording the blood pressure. Femoral vein was cannulated for administration of drug. Trachea was cannulated to record respiration by Mary's tambour. Scoparone and its congeners were injected in 10 mg/kg dose i.v. and effect on blood pressure, its on-set of action and duration was recorded (table). The data represents the mean values of 3–5 experiments.

Discussion. Results are shown in the table. The optimum substitution pattern seemed to be one represented by **I**. An interesting finding, as regards the minimum structural requirement for antihypertensive activity, was obtained in the unsubstituted coumarin itself. The parent dihydroxycoumarin **X** and **XII** showed hypotensive effect but methylation resulted in diminishing the activity of the former but enhancing that of the latter. Further, increase in the size of the alkyl substituent resulted in the decrease in the potency, whereas allyl substitution on oxygen function substantiated the activity. The presence of electronegative groups, NO_2, Cl, Br, on C-3 in **XII**, **XIV**, **XV** potentiated the activity, whereas methyl group on C-4 diminished it. These observations suggest that oxygen function on adjacent carbon (C-6 and C-7) are mandatory, thus offering a flat site which appropriates in such a way that the anionic site of lactone ring connects itself with the receptor site. Surprisingly the maximal duration was observed with dimethyl ether. It may, therefore, be assumed that alkylation in general and methylation in particular, plays an important role, either in deactivating the enzyme or in solubilising the alkyl ether at the hydrophobic site, and subsequently dealkylating the substrate through the biological processes. The mechanism still remains obscure.

Hypotensive activity of dihydroxy coumarins

No.	R^1	R^2	R^3	R^4	R^5	Fall in blood pressure (%)*	Duration of action (min)*
I	H	H	H	OCH_3	OCH_3	58.6±8	160±20
III	H	H	H	H	H	29.16±6	10±3
IV	H	H	H	H	OH	10.0±10	7+2
V	H	H	H	H	OCH_3	13.04±7	5+2
VI	H	H	H	H	C_3H_5	17.4±6	5±3
VII	H	H	H	H	CO_2CH_3	43.4±9	15+3
VIII	H	H	H	H	$CO_2C_6H_5$	00+6	00
IX	H	CH_3	H	H	OH	14.3±7	5+2
X	H	H	OH	H	OH	53.5±15	20+4
XI	H	H	OCH_3	H	OCH_3	9.1±3	5+2
XII	H	H	H	OH	OH	24.0±11	7+3
XIII	H	H	H	OC_2H_5	OC_2H_5	40.7±17	20+6
XIV	H	H	H	OC_3H_7	OC_3H_7	3.7±2	7.0+5
XV	H	H	H	C_3H_5	C_3H_5	23.08+13	30+9
XVI	H	H	H	CO_2CH_3	CO_2CH_3	Nil±	Nil –
XVII	H	H	H	$CO_2C_5H_6$	$CO_2C_6H_5$	Nil	Nil –
XVIII	H	H	H	OC_7H_7	OC_7H_7	Nil	Nil –
XIX	H	H	H	3,5-dinitro-salicyl	3,5-dinitro-salicyl	Nil	Nil –
XX	H	H	H	OCH_3	OH	Nil	Nil
XXI	H	H	H	OH	OCH_3	Nil	Nil
XXII	H	H	H	NO_2	OCH_3	51.0±20	20±6
XVIII	Cl	H	H	OCH_3	OCH_3	68.0±7	25±6
XXIV	Br	H	H	OCH_3	OCH_3	50.0+3	35±10
XV	H	CH_3	H	OH	OH	Nil	Nil
XVI	H	CH_3	H	OCH_3	OCH_3	Nil	Nil
II	L-α-Methyl dopa					12.4±6	120±30

*Values: mean ± SD.



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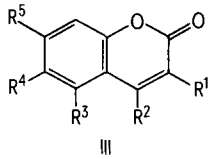

III

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