showed the secondary >C*H*-OH proton at δ 3.30 (J = 12, 4Hz) and 2 sharp methyl singlets at δ 1.16 and 1.19. The absolute configuration of **5** was established by the method of Nakanishi[7]. The CD maxima after addition of Eu(Fod)3 were not well defined at room temperature. However there was a large increase in intensity upon cooling. The longest wavelength maximum at 313.8 nm was strongly negative, $\Delta\varepsilon$ –60.5 (0.74 g/l, –90°C) which established the configuration of **5** at C13 as R. The side chain of 24(R), 25-dihydroxycholesterol, an analogous situation, showed $\Delta\varepsilon$ –11 at 308 nm[8]. Thus the absolute configuration of **3** was R.

A racemic synthetic sample of **3** had slight JH activity[3].

A minor constituent, 6, 10, 14-trimethylpentadeca-5, 9-diene-2, 13-dione (**4**), was isolated as a chromatographically homogenous oil and the formula $C_{18}H_{30}O_2$ established by high resolution MS with major fragment ions at m/e 260 ($M^+ - H_2O$), 192, 125, 97, 71 ($(CH_3)_2$–CH–C≡O$^+$, and 43 (CH_3–C≡O$^+$, base peak).

The presence of 2 ketone groups and 2 double bonds was established by ^{13}C-NMR which showed low field resonances at δ 214.3 (s), 208.6 (s), 136.1 (s), 133.8 (s), 124.5 (d) and 122.7 (d). The ^{1}H-NMR showed the presence of a methyl ketone (δ 2.08) and an isopropyl ketone [δ 1.02 (6H, d, J = 7Hz), 2.44 (1H, heptet, J = 7Hz] together with other resonances at δ 5.02 (2H, m), 2.5–1.9 (12H, m) and 1.56 (6H, bs) which was entirely consistent with the proposed structure **4**. When **3** was treated in benzene with strong mineral acids or boron triflouride etherate a complex mixture of cyclic alcohols was formed but no **4** could be detected in this mixture by TLC. It is therefore unlikely that **4** is an artefact by acid catalysed rearrangement of **3**.

The presence of such biologically active compounds in the brown alga *Cystophora moniliformis* is perhaps a chemical defence against predation and is probably analogous to the occurrence of many insect JH and moulting hormone active compounds in vascular plants.

Constituents of propolis

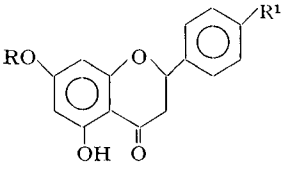
E. L. Ghisalberti, P. R. Jefferies, R. Lanteri and J. Matisons[1]

Department of Organic Chemistry, University of Western Australia, Nedlands (W. A. 6009, Australia), 18 July 1977

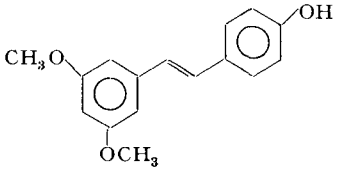
Summary. The major constituents of propolis collected in Western Australia have been isolated and identified as pterostilbene (**5**), xanthorrhoeol (**6**), sakuranetin (**2**) and pinostrobin (**1**).

In recent years there has been renewed interest in the composition of propolis, the resinous substance collected by bees from various plant sources. Bees use propolis for coating hive parts and the cell interiors of the honeycomb and also to seal cracks and crevices in the hive. Propolis has been used for a long time in folk medicine particularly for the cure of respiratory disorders[2], treatment of dermatoses and burns[3] and as a local anaesthetic[4]. Despite numerous reports[5] of the apparent pharmacological activity of extracts of propolis, a limited amount of work has been done on the isolation of the constituents. From samples of European propolis a number of simple aromatic compounds, flavones, flavonols and flavanones have been isolated[6–8]. We have investigated the chemical constituents of Western Australian propolis and the isolation and identification of the major components is the subject of this report.

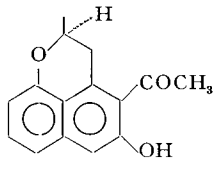
Propolis[9] (300 g) was extracted with 70% aqueous ethanol and the fraction obtained was dissolved in ether and partitioned into 5% HCl, saturated $NaHCO_3$, 10% Na_2CO_3 and 5% NaOH solutions. The major part (90%) of the material extracted (40 g) was in the NaOH fraction and the remainder was divided almost equally between the other 4 fractions. Apart from the neutral fraction, which appeared to contain mostly fats and sterols, and the $NaHCO_3$ fraction, mostly flavones, all the other fractions were examined in detail. For the isolation and purification of the constituents of each fraction column, analytical and



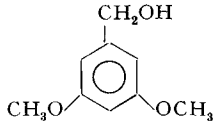
	R	R¹
1	CH_3	H
2	CH_3	OH
3	H	OCH_3
4	CH_3	OCH_3



5



6



7

1 Acknowledgments. We gratefully acknowledge Dr H. Duewell of the University of Newcastle, New South Wales, for an authentic sample of xanthorrhoeol.

2 J. F. Thorpe and M. A. Whiteley (ed.), Thorpe's Dictionary of Applied Chemistry, vol. X, 4th ed., p. 227. Longmans, Green and Co., 1950.

3 L. A. Lindenfelser, Am. Bee J. *107*, 90 and 130 (1967) and references therein.

4 Ves. Todorov, St. Drenovski and V. Vasilev, Farmatsiya, Sofia *18*, 23 (1968) (Chem. Abstr. *70*, 11366k [1969]).

5 A. P. Walker, Annotated Bibliography on Propolis, 1976, International Bee Research Association, Bibliography No. 16.

6 J. Cizmarik and I. Matel, Experientia *26*, 713 (1970), and J. Apicult. Res. *12*, 52 (1973).

7 S. A. Propavko, Apicult. Abstr. *21*, 127 (1970) (Chem. Abstr. *74*, 10749t [1971]).

8 E. M. Schneidewind, H. Kala, B. Linzer and J. Metzner, Pharmazie *30*, 803 (1975).

9 The sample of propolis was collected in the Mandurah region in the southwest of Western Australia.

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].

10 T. J. Mabry, K. R. Markham and M. B. Thomas, The Systematic Identification of Flavonoids, Springer-Verlag, 1970.
11 H. Audier, Bull. Soc. Chim. France *1966*, 2892.
12 G. Lindstedt, Acta. chem. scand. *4*, 1042 (1950) (Chem. Abstr. *45*, 2484e [1951]).
13 F. M. Dean, Naturally Occurring Oxygen Ring Compounds, p. 357. Butterworths, London 1963.
14 B. A. Bohm, Phytochemistry *7*, 1687 (1968).
15 E. Spath and J. Schlager, Chem. Ber. *73*, 881 (1940).
16 H. Duewell, Aust. J. chem. *18*, 575 (1965).
17 A. J. Birch and C. J. Dahl, Aust. J. Chem. *27*, 331 (1974).
18 W. E. Hillis and M. Hasegawa, Biochem. J. *83*, 503 (1963).
19 T. R. Seshadri, Phytochemistry *11*, 891 (1972).
20 H. Lyr, Enzymologia *23*, 231 (1961).
21 R. M. V. Assumpcao, S. M. Koop and O. R. Gottleib, Anais Acad. bras. cienc. *40*, 297 (1968) (Chem. Abstr. *71*, 46664p [1969]).
22 H. Yasuo, Y. Arimoto and T. Misato, Japan Kokoi *75*, 111, 230 (1975). (Chem. Abstr. *84*, P13498c [1976]).
23 Société de Recherches Industrielles 'SORI' Fr. *1*, 578, 715 (1969) (Chem. Abstr. *72*, P111302f [1970]).

Hypotensive activity of some dihydroxycoumarins and their congeners

R. S. Thakur, Suresh C. Bagadia and M. L. Sharma

Regional Research Laboratory, Jammu-180001 (India), 30 August 1976

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.