THREE FLAVANONES FROM LEAVES OF *EUCALYPTUS SIEBERI*

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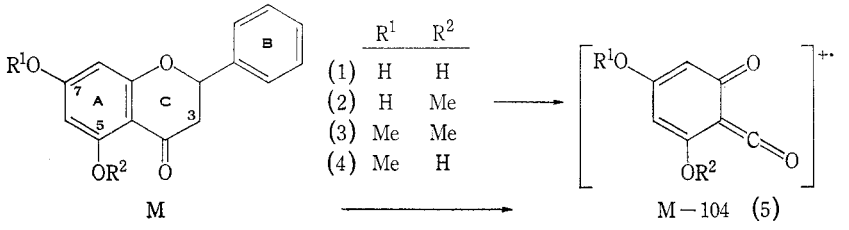
In a chemotaxonomic survey[1] by paper chromatography of the occurrence of polyphenols in eucalypt leaves, a spot due to an unidentified substance (unknown compound D) was observed in several species including *E. sieberi* L. Johnson. We have reexamined this species and from a leaf extract isolated three closely related substances with chromatographic behaviour similar to compound D, which have properties corresponding to flavanones. In particular, all three compounds showed a dull red colour when sprayed with sodium borohydride followed by acid fuming.[2] The i.r. spectra showed the presence of carbonyl groups, confirmed by reaction with dinitrophenylhydrazine; the u.v. spectra, with strong peaks around 286 nm, were consistent with those of flavanones, and likewise the mass spectra,[3] which furthermore had strong M—77 ions indicating unsubstituted B rings. The latter inference was supported by five-proton multiplets in the aromatic region of the n.m.r. spectra, and



by characteristic i.r. peaks around 765 and 700 cm^{-1}. All three compounds were reduced by strong acid and magnesium but not zinc: this indicated they had no 3-hydroxyl groups,[4] the absence of which was confirmed by the M—104 ions (5) which formed the base peaks in their mass spectra.

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The compound of lowest molecular weight, which was present in least amount, reacted with ferric chloride and coupled with diazotized *p*-nitroaniline.[1] Its u.v. spectrum showed considerable bathochromic shifts with aluminium chloride[5] and with sodium acetate,[5] indicating the presence of hydroxyl groups at positions 5 and 7 respectively. The broad i.r. band around 3120 cm^{-1} and the carbonyl absorption at 1629 cm^{-1} were in accord with the presence of a 5-hydroxyl group, and the data indicated that the compound was pinocembrin (1); its identity was confirmed by a comparison of its properties with those of a synthetic specimen.

The second flavanone also gave a ferric chloride test, but coupled sluggishly with diazotized *p*-nitroaniline;[1] its u.v. spectrum showed a bathochromic shift with sodium acetate[5] but not with aluminium chloride.[5] These data indicated that the compound had a 7-hydroxyl but no 5-hydroxyl group, consistent with the presence of a methoxyl proton resonance in the n.m.r. spectrum, and with an i.r. hydroxyl absorption at 3475 cm^{-1} and a carbonyl at 1660 cm^{-1}. The substance thus has the structure of alpinetin[6] (2), a rather rare flavanone not previously encountered in eucalypts. Its melting point agreed with that reported for alpinetin, but was over 80° higher than that of the isomeric pinostrobin[7a] (4); moreover, an authentic sample of (4) showed a u.v. shift with aluminium chloride but not with sodium acetate. The identity of (2) was confirmed by conversion into its *O*-methyl derivative, which proved identical with the *O,O*-dimethyl derivative (3) prepared from pinocembrin.

The third flavanone gave none of the phenolic tests or u.v. shifts described for (1) or (2), but it had an i.r. carbonyl absorption at 1671 cm^{-1}, and its n.m.r. spectrum showed two methoxyl proton resonances with benzene shifts corresponding to locations at positions 5 and 7.[8] Its identity as *O,O*-dimethyl pinocembrin (3), which does not appear to have been found previously in nature, was confirmed by a comparison of its properties with those of an authentic sample prepared from (1).

Experimental

Melting points are uncorrected. Ultraviolet spectra were run on a Perkin–Elmer 400A Spectracord in methanol unless otherwise stated; infrared spectra were determined with a Perkin–Elmer 221 spectrophotometer in Nujol mulls; proton magnetic resonance spectra were recorded on a Jeolco JNM4H-100 spectrometer at 100 MHz for *c.* 5% solutions in DMSO-d_6 with HMDS or TMS as external standard unless otherwise stated. Mass spectra were measured on an AEI MS9 instrument at 70 eV. Paper chromatography was carried out on Whatman No. 1 paper using either n-butanol : acetic acid : water 6 : 1 : 2 (BAW) or 6% aqueous acetic acid (HOAc) as solvent; polyphenol spots were visualized by spraying with aqueous $FeCl_3$ (1%)–$K_3Fe(CN)_6$ (1%) or more specifically, with diazotized *p*-nitroaniline (0·05%) in 20% aqueous sodium acetate. Thin-layer chromatography was carried out on Camag silica, using either chloroform : ethyl acetate : formic acid 7 : 4 : 1 (CEF) or methanol : chloroform : light petroleum 2 : 4 : 7 (MCP) as solvent systems, and antimony trichloride (10% in $CHCl_3$) or diazotized *p*-nitroaniline as visualizing agents.

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Isolation of Flavanones

Dried milled leaves of *E. sieberi* (1 kg), collected at Fingal, Tasmania, were exhaustively extracted with methanol and the extract, concentrated under vacuum to *c.* 1 l., was poured slowly into *c.* 6 l. of vigorously stirred water. The green waxy precipitate was filtered off, redissolved in methanol, and reprecipitated as before. The procedure was repeated twice more, after which no polyphenols could be detected in the green precipitate by paper chromatography. The combined aqueous methanolic solutions were extracted with light petroleum (40–60°) until no further chlorophyll or wax was removed, then concentrated under vacuum to 1·5 l. and extracted continuously in a liquid–liquid extractor with ether for several days. The ether extract was evaporated in vacuum to 500 ml and allowed to stand in the cool-room. The off-white precipitate which settled appeared by t.l.c. to be a mixture of the three flavanones (1), (2), and (3) together with minor components. They were separated by preparative t.l.c. using solvent systems CEF and MCP, and further amounts were obtained similarly from the mother liquor. Total approximate yields: (1), 500 mg (0·05%); (2), 100 mg (0·01%); (3), 50 mg (0·005%).

Pinocembrin (*1*)

Pinocembrin had R_F 0·91 in BAW, 0·05 in HOAc, 0·86 in CEF, and 0·68 in MCP. It crystallized from methanol in short white needles, m.p. 192–193° (lit.[7b] 194–195°), undepressed on admixture with synthetic pinocembrin, and had $[\alpha]_D^{15}$ −45·3° (*c*, 0·9 in acetone) (lit.[9] −46°); λ_{max} 289, 324sh nm, $\log \epsilon_{max}$ 4·33, 4·02; λ_{max} in MeOH/$AlCl_3$: 310, in MeOH/NaOAc: 323 nm; δ 3·07 (H 3*eq*, q), 3·52 (H 3*ax*, q), 5·90 (H 2, q), 6·28 (H 6+H 8, s), 7·80 (ring-B H, m); 11·20, 12·48 (7-OH, m, 5-OH, s, removed with D_2O); $J_{2,3eq}$ 3·0, $J_{2,3ax}$ 13·0, $J_{3eq,3ax}$ 17·0 (Found: C, 70·1; H, 4·7. Calc. for $C_{15}H_{12}O_4$: C, 70·3; H, 4·7%).

On methylation with methanolic dimethyl sulphate and potassium carbonate, (1) gave *O,O*-dimethylpinocembrin (3), m.p. 156–157°.

Alpinetin (*2*)

Alpinetin had R_F 0·87 in BAW, 0·04 in HOAc, 0·60 in CEF, and 0·62 in MCP. It crystallized from acetone in short white needles, m.p. 218–220° (lit.[7] 223°), and had $[\alpha]_D^{15}$ −46·0 (*c*, 0·9 in $CHCl_3$); λ_{max} 285, 322sh nm, $\log \epsilon_{max}$ 4·37, 3·94; λ_{max} in MeOH/NaOAc: 321 nm; δ 2·78 (H 3*eq*, q), 3·16 (H 3*ax*, q), 3·92 (MeO, s), 5·66 (H 2, q) 6·16 (H 6, d), 6·24 (H 8, d), 7·63 (ring-B H, m), 11·20 (7-OH, m, removed by D_2O); $J_{2,3eq}$ 3·2, $J_{2,3ax}$ 13·1, $J_{3eq,3ax}$ 17·0, $J_{6,8}$ 3·0 (Found: C, 71·1; H, 5·3. Calc. for $C_{16}H_{14}O_4$: C, 71·1; H, 5·2%).

On methylation with diazomethane in DMSO, (2) gave *O,O*-dimethylpinocembrin (3), m.p. 155–156° undepressed on admixture with a synthetic sample.

O,O-*Dimethylpinocembrin* (*3*)

Isolated as described above from *E. sieberi* extracts, (3) had R_F 0·94 in BAW, 0·07 in HOAc, 0·75 in CEF, and 0·79 in MCP. It crystallized from acetone in long white needles, m.p. 159–160° undepressed by admixture with a synthetic sample, and had $[\alpha]_D^{15}$ −45·8° (*c*, 2·0 in 50% MeOH/$CHCl_3$); λ_{max} 283, 322sh nm, $\log \epsilon_{max}$ 4·39, 3·66; δ ($CDCl_3$, TMS) 2·77 (H 3*eq*, q), 3·02 (H 3*ax*, q), 3·74 (7-MeO, s), 3·89 (5-MeO, s), 5·40 (H 2, q), 6·10 (H 6, d), 6·18 (H 8, d), 7·42 (ring-B H, s); $J_{2,3eq}$ 3·2, $J_{2,3ax}$ 13·0, $J_{3eq,3ax}$ 17·0, $J_{6,8}$ 3·0. Δδ($CDCl_3$–C_6D_6): +0·43 (5-MeO), +0·64 (7-MeO) (Found: C, 72·0; H, 5·6. $C_{17}H_{16}O_4$ requires C, 71·8; H, 5·7%).

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