Phytochemistry, 1974, Vol. 13, pp. 853 to 855. Pergamon Press. Printed in England.

COUMARINS OF *SKIMMIA JAPONICA*

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(*Received* 19 *October* 1973. *Accepted* 1 *November* 1973)

Key Word Index—*Skimmia japonica*; Rutaceae; coumarins; (+)-oxyneucedanin methanolate; taraxerol.

Abstract—The coumarins, isoimperatorin, isomeranzin, meranzin hydrate, oxypeucedanin, oxypeucedanin hydrate, oxypeucedanin methanolate and umbelliferone and the triterpene, friedoolean-14-en-3β-ol (taraxerol) were isolated from leaves of *Skimmia japonica*.

IN PREPARATION for a study of the biosynthesis of furoquinolines,[1] furocoumarins and related prenyl derivatives, we investigated systematically the leaf constituents of the garden shrub *Skimmia japonica*, growing locally. Tertiary and quaternary alkaloids of *S. japonica* were reported earlier,[2] and we now describe the isolation and identification of some other components.

Extraction of the dry leaves with methanol and chromatography of the solvent-free extract on alumina furnished the pentacyclic triterpene, friedoolean-14-en-3β-ol (taraxerol, skimmiol)[3] (0·17%) and the well-known coumarins, umbelliferone (0·008%), isoimperatorin (**1a**) (0·30%), (+)-oxypeucedanin (**1b**) (0·08%) and (+)-oxypeucedanin hydrate (**1c**) (0·028%). A new coumarin, (+)-oxypeucedanin methanolate (**1d**) (0·04%) was also obtained. Its structure was indicated by the NMR spectrum, which in addition to furocoumarin resonances showed a two-proton multiplet at τ 5·05 (-OC$\underline{H}_2$-C-), a one-proton signal at τ 6·04 (-C$\underline{H}$(OH)-), a three-proton singlet at τ 6·72 (-OC$\underline{H}_3$) and a six-proton singlet at τ 8·72 (-C(OCH$_3$)Me$_2$). (+)-Oxypeucedanin (**1b**) was converted into (+)-oxypeucedanin methanolate (**1d**) by treatment with boron trifluoride in methanol, a reaction which probably occurs with retention of configuration at the chiral centre and indicates the (*R*)-configuration for the methanolate as shown. Friedoolean-14-en-3β-ol, umbelliferone, isoimperatorin, oxypeucedanin and other coumarins have been obtained previously from *Skimmia* species,[4-9] but not the hydrate (**1c**) and the methanolate (**1d**). These oxypeucedanin derivatives may be artifacts, but we consider this unlikely, since (+)-oxypeucedanin is unaffected in the conditions used for extraction and for separation of constituents.

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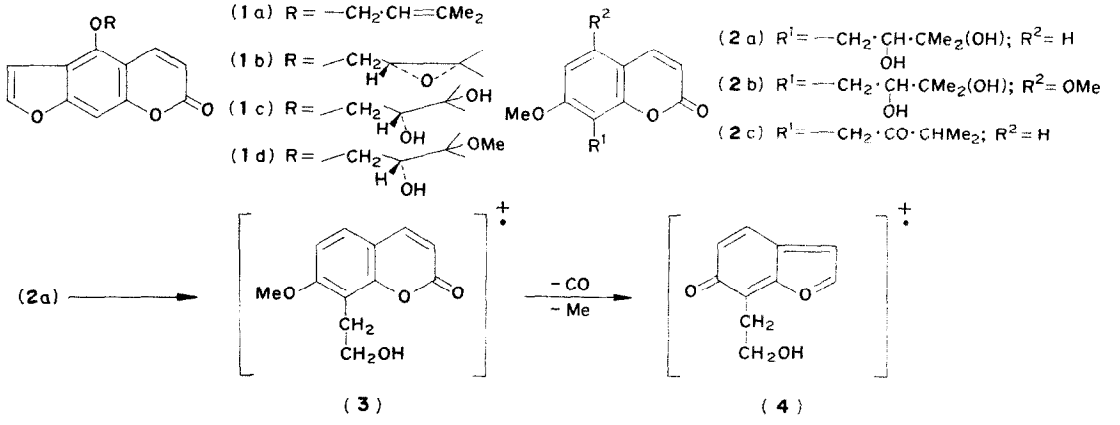
The alkaloids of *S. japonica* were separated with aqueous acid, and further investigation of this fraction resulted in the isolation of two coumarins. One compound, $C_{15}H_{18}O_5$, was clearly the diol, (—)-meranzin hydrate (**2a**). Thus, there was IR absorption at 3600 cm^{-1} (OH) and the NMR spectrum showed signals characteristic of the dioxygenated prenyl side chain; spin–spin decoupling led to the assignment of doublets at τ 2·7 and 3·1 (*o*-aromatic protons) and at τ 2·37 and 3·75 (*α*-pyrone ring protons). The MS is analogous to that of the related diol, mexoticin (**2b**),[5] with peaks at M-15, M-59 and M-89 indicating successive cleavage of the side-chain and the abundant ions at *m/e* 220 (53%) and 177 (100%) suggesting the fragmentation (**2a**) → (**3**) → (**4**). Meranzin hydrate was obtained first by hydrolysis of the corresponding epoxide, meranzin,[6,7] and more recently was identified with meranzin as a constituent of *Prangos ferulacea* (Umbelliferae).[8]



The second coumarin, $C_{15}H_{16}O_4$, isolated from the aqueous acid extract of *S. japonica* was the optically-inactive ketone, isomeranzin (**2c**). The NMR spectrum was almost identical in the region τ 2·3–4·0 to that of meranzin hydrate and the presence of a 3-methyl-2-oxobutyl side-chain was indicated by the appearance of a two-proton singlet at τ 5·95, a one-proton septet at τ 7·17 and signals at τ 8·74 and 8·81 [$CH(C\underline{H}_3)_2$]. Isomeranzin has not been isolated previously from a natural source, but is obtained by acid-treatment of the epoxide, meranzin,[6] or of meranzin hydrate. Since both reactions require elevated temperatures, isomeranzin apparently is not an artifact formed from the epoxide or the diol. The isolation of meranzin hydrate (**2a**) and isomeranzin (**2c**) from the acid-soluble alkaloid fraction and not by direct chromatography of the methanol extract was unexpected. A possible explanation is that the two compounds occur as water soluble glycosides of *o*-hydroxycinnamic acids that during extraction with aqueous acid and subsequent neutralisation undergo hydrolysis and cyclisation.

EXPERIMENTAL

The NMR spectra were determined with a Varian HR-100 spectrometer using tetramethylsilane as internal standard, and MS with an A.E.I. MS 902 instrument. TLC was with $CHCl_3$ on silica gel G.

Isolation of the constituents. (a) Leaves of *Skimmia japonica* were dried at 30–35° and powdered. The powder (380 g) was extracted with MeOH (3 l.) at 20° for 7 days, and the soln was evaporated at 40–50°.

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The residue was chromatographed on alumina (2500 g), and each constituent was purified by preparative TLC. Elution with petrol. (b.p. 40–60°) furnished skimmiol (0·631 g, 0·17%), plates (from Et_2O), m.p. 282–283° (lit.[9] 279–281°), R_f 0·60, NMR ($CDCl_3$) 4·46 (*dd*, C=C–H) and 6·81 τ (*t*, –CH(OH)–) (Found: M^+ 426·385. Calc. for $C_{30}H_{50}O$: 426·386). Elution with ether gave isoimperatorin (1·126 g, 0·30%), plates (from diisopropyl ether), m.p. 108–109° (lit.[10] 108°), R_f 0·96, $[\alpha]_D$ 0° ($CHCl_3$) (Found: C, 71·4; H, 5·2; M^+ 270·089. Calc. for $C_{16}H_{14}O_4$: C, 71·1; H, 5·2%; 270·089). Elution with Et_2O–$CHCl_3$ (1 : 1) gave oxypeucedanin (0·3 g, 0·08%), needles (from Et_2O), m.p. 102–103° (lit.[10] 104°), R_f 0·88, $[\alpha]_D$ +13·4° ($CHCl_3$) [lit.[10] +13° ($CHCl_3$) lit.[11] +20·1 ($CHCl_3$)] (Found: C, 67·0; H, 5·2; M^+, 286·0833. Calc. for $C_{16}H_{14}O_5$: C, 67·1; H, 4·9%; M^+, 286·084). Elution with $CHCl_3$ furnished *oxypeucedanin methanolate* (**1d**) (0·04 g, 0·011%), separating from acetone-petrol. (b.p. 40–60°) in needles, m.p. 123–125°, R_f 0·40, $[\alpha]_D$ +12° ($CHCl_3$), ν_{max} ($CHCl_3$) 3700 and 1730 cm^{-1}, NMR ($CDCl_3$) 1·77 (1-H, *d*, *J* 10 Hz), 2·40 (1-H, *d*), 2·86 (1H, *s*), 2·98 (1H, *q*), 3·73 (1H, *d*, *J* 10 Hz), 5·05 (2H, *m*), 6·04 (1H, *m*), 6·72 (3H, *s*), 7·16 (broad, removed with D_2O) and 8·72 τ (6H, *s*) (Found: M^+, 318·110. $C_{17}H_{18}O_6$ requires: M^+, 318·110). Elution with EtOAc–MeOH (9 : 1) gave umbelliferone (0·03 g, 0·008%), needles (from $CHCl_3$), m.p. 229–231° R_f 0·18, identical (m.m.p. and IR spectrum) with an authentic sample. Further elution with EtOAc–MeOH (9 : 1) furnished oxypeucedanin hydrate (0·10 g, 0·028%), needles (from Et_2O), m.p. 125–128° (lit.[10] 130–131°), R_f 0·09, $[\alpha]_D$ +16° (Me_2CO) [lit.[11] +18° (Me_2CO)], ν_{max} 3450, 1725 cm^{-1} (Found: C, 63·2; H, 5·6; M^+, 304·095. Calc. for $C_{16}H_{16}O_6$: C, 63·2; H, 5·3%; M^+, 304·095). (b) The constituents of *S. japonica* leaves obtained by acid treatment were chromatographed on alumina and 2 new components were isolated. Elution with petrol. (b.p. 40–60°)–Et_2O (1 : 1) furnished isomeranzin (**2c**) (0·017%), prisms (from pentane), m.p. 60–62° (lit.[6] m.p. 66°), $[\alpha]_D$ 0° ($CHCl_3$), λ_{max} (MeOH) 259 (ε 2860), 280 (ε 2750) and 326 mμ (ε 8810), NMR ($CDCl_3$) 2·30 (1H, *d*, *J* 10 Hz), 2·57 (1H, *d*), 3·12 (1H, *d*), 3·76 (1H, *d*, *J* 10 Hz), 5·95 (2H, *s*), 6·1 (3H, *s*), 7·17 (1H, septet), 8·74 (3H, *s*) and 8·81 τ (3H, *s*). It was identical (m.m.p. and IR) with a sample prepared from the hydrate (see below). Elution with chloroform gave meranzin hydrate (**2a**) (0·06%), prisms (from diisopropyl ether), m.p. 131–132° (lit.[7] m.p. 128°), $[\alpha]_D$ −57° (EtOH), −29° ($CHCl_3$) [lit.[8] −53 (EtOH)], NMR ($CDCl_3$) 2·37 (1H, *d*), 2·7 (1H, *d*), 3·1 (1H, *d*), 3·75 (1H, *d*), 6·1 (3H, *s*), 6·3 (1H, *m*), 6·9 (2H, *m*), 7·33 (2H, broad *s*, disappears on addition of D_2O) and 8·65 τ (6H, *s*). *m/e* 278 (M^+), 263 (6%), 220 (53), 219 (25), 190 (27), 189 (26) and 177 (100) (Found: C, 64·3; H, 6·4. Calc. for $C_{15}H_{18}O_5$: C, 64·8; H, 6·5%).

Interconversions. (a) Hydrolysis of oxypeucedanin, $[\alpha]_D$ +13·4°, by refluxing with 1% aq. oxalic acid furnished oxypeucedanin hydrate, $[\alpha]_D$ +20°. (b) A soln of oxypeucedanin (99 mg) in MeOH (10 ml) containing BF_3 etherate (2 drops) was kept for 24 hr and added to aq. HCl. $CHCl_3$ extraction and purification of the products by preparative TLC on silica gel gave oxypeucedanin methanolate (needles from acetone–petrol.) (82 mg), m.p. and m.m.p. 123–125°, $[\alpha]_D$ +16·5° ($CHCl_3$). Oxypeucedanin was unaffected by prolonged contact with MeOH or with MeOH and alumina. (c) Meranzin hydrate (0·14 g) was refluxed with 20% aq. H_2SO_4 (20 ml) for 12 hr. Chromatography of the product on alumina and elution with Et_2O–petrol. (3 : 7) gave isomeranzin (0·08 g) (from H_2O), m.p. 68–71° (Found: C, 69·4; H, 6·5. Calc. for $C_{15}H_{16}O_4$: C, 69·2; H, 6·2%).

Acknowledgement—A research grant was provided by the Science Research Council.

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