South African Plant Extractives. Part III.[1] Helichrysin, a New Chalcone Glucoside from a *Helichrysum* Species

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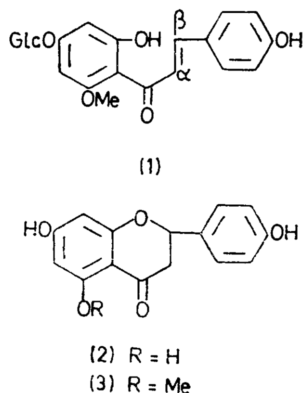
Luteolin 7-glucoside, (−)-2-*O*-methylchiroinositol, and a new chalcone glucoside, helichrysin (1), have been isolated from the flowers of a *Helichrysum* species.

THE genus Helichrysum (Compositae) is recorded as having *ca.* 500 species, 200 of them (' everlasting flowers ') in South Africa. The chemistry of the flowers of a variety identified as being close to *H. cooperi* has now been investigated.

An acetone extract of the flowers gave the known luteolin 7-glucoside, (−)-2-*O*-methylchiroinositol, and a new glycoside, helichrysin, m.p. 246° (decomp.) ($C_{22}H_{29}O_{10}$, 2 H_2O), $\lambda_{max.}$ (MeOH) 360 nm, containing one methoxy-group [τ 6.12 (3 H, s)]. Helichrysin gave a pale yellow spot on a chromatogram, turning orange with ammonia vapour, which indicated that it was a chalcone derivative.[2]

Acidic hydrolysis of helichrysin gave three products: glucose (identified by paper chromatography and by the specific reaction with glucose oxidase test reagent), a chalcone aglycone, helichrysetin, m.p. 328—330° (decomp.), $\lambda_{max.}$ (MeOH) 358 nm, corresponding to 360 nm for helichrysin, placing the sugar in ring A,[3] and a flavanone $C_{16}H_{14}O_5$, m.p. 265°.

The u.v. absorption of the flavanone was similar to that



(1)

(2) R = H
(3) R = Me

of naringenin (2), indicating a phloroglucinol-derived ring A in the chalcone. The wavelength ($\lambda_{max.}$ 284 nm; *cf.* 289 nm for naringenin) shows a difference in the A rings. There was a small shift to 281 nm on addition of aluminium chloride; a compound with a free 5-hydroxy-group shows a large positive shift with this reagent.[4] The compound was therefore 5-*O*-methylnaringenin (3).

The position of the sugar in helichrysin was determined by methylation with dimethyl sulphate, giving a product of m.p. 178° (decomp.). This was shown to be fully methylated by the lack of a spectral shift on the addition of alkali to the ethanolic solution. Acidic hydrolysis then gave a methylated chalcone, m.p. 125°, $\lambda_{max.}$(MeOH) 333 nm, shifted to 390 nm on addition of sodium acetate, indicating a free 4'-OH group.[5] This chalcone did not cyclise to a flavanone with acid and must therefore be methylated at the 2'- and 6'-OH groups, which confirms that the free OH group, and hence the glucose residue in helichrysin, is at position 4'. Helichrysin therefore has structure (1) and is 6'-*O*-methylchalcononaringenin 4'-glucoside. No evidence for the stereochemistry of the glucoside linkage has been obtained.

EXPERIMENTAL

N.m.r. spectra were recorded with a Varian T60 spectrometer for solutions in dimethyl sulphoxide (internal Me_4Si standard). M.p.s were determined with a Kofler hot-stage apparatus. U.v. spectra were recorded for solutions in methanol (or 95% ethanol when too sparingly soluble).

Extraction.—Helichrysin. Helichrysum flowers collected at Umzinto, near Durban, were identified as *Helichrysum* ps. aff. *H. cooperi* Harv. by Dr. O. M. Hilliard of the Natal University Herbarium, Pietermaritzburg. These were dried, milled, and extracted successively with hexane, ether, and acetone. The acetone extract was evaporated and the residue (3.5 g) boiled with water. On cooling, the filtered solution gave yellow crystals (1 g) which after several recrystallisations gave *helichrysin* (78 mg), m.p. 246° (decomp.) (from methanol) [Found: C, 54.7, 54.7; H, 5.05, 5.1%; *m/e* (base peak), 286.0828. $C_{22}H_{24}O_{10},2H_2O$ requires C, 54.5; H, 5.8%. $C_{16}H_{14}O_5$ requires *m/e* 286.0841]; M^+ 448, $\lambda_{max.}$ (MeOH) 236, 272, 296sh, and 360 nm (log ε 4.18, 4.12, 4.05, and 4.31), $\lambda_{max.}$ (MeOH–NaOMe) 242sh, 269, 302sh, and 382 nm (log ε 4.26, 4.29, 3.98, and 4.30), $\lambda_{max.}$ (MeOH–$AlCl_3$) 246sh, 274sh, 294sh, 322sh, 350sh, 370sh, and 396 nm (log ε 4.43, 4.34, 4.30, 4.29, 4.32, 4.38, and 4.42), $\lambda_{max.}$ (MeOH–$AlCl_3$–HCl) 250sh, 274sh, 294sh, 322sh, 342sh, 362sh, and 386 nm (log ε 4.46, 4.38, 4.36, 4.34, 4.37, 4.40, and 4.44), $\lambda_{max.}$ (MeOH–NaOAc) 270, 302sh, 322sh, 374, and 446sh nm (log ε 4.48, 4.37, 4.36, 4.44, and 4.29), $\lambda_{max.}$ (MeOH–NaOAc–H_3BO_3) 270, 286, and 350 nm (log ε 4.28, 4.20, and 4.28); τ 2.30 (1 H, s, H-β), 2.40 (1 H, s, H-α), 2.50 (1 H, q, H-2 $J_{2,6}$ 2, $J_{2,3}$ 8 Hz), 2.65 (1 H, q, H-6, $J_{6,2}$ 2, $J_{6,5}$ 8 Hz), 3.07 (1 H, q, H-3 $J_{3,5}$ 2, $J_{3,2}$ 8 Hz), 3.20 (1 H, q, H-5, $J_{5,3}$ 2 $J_{5,6}$ 8 Hz), 3.47 (1 H, d, H-3', $J_{3',5'}$ 2 Hz), 3.74 (1 H, d, H-5 $J_{5',3'}$ 2 Hz), 5.00 (1 H, s, glucosyl H-1), and 6.12 (3 H, s, OMe).

(−)-2-O-*Methylchiroinositol.* A cold water extract of the residue from acetone extraction was taken nearly to dryness *in vacuo.* (−)-2-*O*-Methylchiroinositol crystallised from the residue and was isolated with the aid of a little acetone. Sublimation gave diamond-shaped crystals, m.p. 195°, identified by *X*-ray crystallography (space group $P2_1$, a = 8.7, b = 7.2, c = 6.7 Å, β = 90°).[6]

[1] Part II, K. H. Pegel and W. G. Wright, *J. Chem. Soc.* (*C*), 1969, 2327.

[2] J. B. Harborne, ' Comparative Biochemistry of the Flavonoids,' Academic Press, London, 1967, p. 78.

[3] Ref. 2, p. 82, table 3.3.

[4] Ref. 2, p. 90.

[5] T. J. Maybry, K. R. Markham, and M. B. Thomas, ' Systematic Identification of Flavanoids,' Springer-Verlag, Berlin, 1970, p. 228.

[6] ' Crystal Data Determinative Tables,' U.S. Department of Commerce, Washington, 3rd edn., vol. 1, p. M–97.

Luteolin 7-glucoside. The residue from the first crystallisation of helichrysin was extracted with ethyl acetate; evaporation gave yellow crystals of luteolin 7-glucoside, m.p. 256°, identical (m.p., colour tests, u.v., and ^{1}H n.m.r. data) with an authentic sample.

Hydrolysis of Helichrysin.—Helichrysin (10 mg), 6% hydrochloric acid (25 ml), and enough methanol to cause complete dissolution of the chalcone were heated on a water-bath for 1 h, cooled and set aside in a refrigerator overnight. *Helichrysetin* (6′-O-*methylchalcononaringenin*) separated from the solution and crystallised from methanol in pale yellow needles (4 mg), m.p. 328—330° (decomp.). Testing with aniline acetate showed the absence of carbohydrate, and the colours in visible light and under u.v. light were the same as those for helichrysin, showing that the aglycone still had a chalcone structure (Found: M^+, 286.0828. $C_{16}H_{14}O_5$ requires *M*, 286.0841) $\lambda_{max.}$ (MeOH) 240sh, 256sh, 270, 298sh, and 358 nm, $\lambda_{max.}$ (MeOH–NaOMe) 274, 330sh, and 408 nm, $\lambda_{max.}$ (MeOH–$AlCl_3$) 210, 232sh, 272, 298sh, 324sh, 374sh, and 416nm, $\lambda_{max.}$ (MeOH–$AlCl_3$–HCl) 198, 232sh, 260sh, 273sh, 294sh, 324sh, and 360 nm, $\lambda_{max.}$ (MeOH–NaOAc) 266, 320sh, 386, and 430sh nm, $\lambda_{max.}$ (MeOH–NaOAc–H_3BO_3) 255 and 368 nm. Extraction of the aqueous residue with ether gave 5-O-*methylnaringenin* (2 mg), m.p. 265° (from ether) (Found: C, 67.2; H, 5.0%; M^+, 286. $C_{16}H_{14}O_5$ requires C, 67.1; H, 4.9%; *M*, 286), $\lambda_{max.}$ (MeOH) 284, 326sh, and 370sh nm, $\lambda_{max.}$ (MeOH–NaOMe) 246, 280sh, and 320 nm, $\lambda_{max.}$ (MeOH–$AlCl_3$) 281, 320sh, and 364 nm, $\lambda_{max.}$ (MeOH–$AlCl_3$–HCl) 282, 320sh, and 362 nm, $\lambda_{max.}$ (MeOH–NaOAc) 284sh, 322, and 380sh nm, $\lambda_{max.}$ (MeOH–NaOAc–H_3BO_3) 284 and 332sh nm. The aqueous residue from another, similar, hydrolysis was extracted with ethyl acetate to remove aglycones and then carefully neutralised with aqueous sodium hydroxide. The residue obtained by evaporation of the solvent and thorough drying was extracted with hot dry pyridine. The solvent was removed and the small residue dissolved in three drops of water. This solution reacted positively to two different test papers employing glucose oxidase as a specific test for glucose (Clinistix, Ames Co., and Test Tape, Lilly Laboratories).

Methylation of Helichrysin.—Dimethyl sulphate (0.5 ml), helichrysin (117 mg), and anhydrous potassium carbonate in dried acetone (170 ml) were refluxed until the yellow colour had disappeared, and then set aside overnight. The potassium carbonate was filtered off and washed once with acetone. The colourless solution was evaporated under high vacuum, and methanol was added to the residue. Pale yellow crystals separated. Recrystallisation from methanol gave the methylated compound (16.7 mg), m.p. 178° (decomp.), $\lambda_{max.}$ (EtOH) 243, 266, 284sh, and 330 nm (not changed with addition of sodium methoxide). Under u.v. light, a solution in ethanol gave a brilliant fluorescent blue spot, which was unchanged on exposure to ammonia.

4′-Hydroxy-2′,4,6′-tri-O-methylchalcononaringenin.—The methylated helichrysin (16.7 mg) was heated on a water-bath with hydrochloric acid (6%; 40 ml), with addition of ethanol until complete dissolution was achieved. Heating was continued for 7 h, and the solution was then set aside for [illegible] days. Extraction with ether and crystallisation from methanol gave fine long needles, m.p. 125°, $\lambda_{max.}$ (MeOH) 243, 266, 300sh, and 333 nm, $\lambda_{max.}$ (MeOH–NaOMe), 243sh, 256, 295sh, and 395 nm, $\lambda_{max.}$ (MeOH–$AlCl_3$) 243, 266, 300sh, and 334 nm, $\lambda_{max.}$ (MeOH–$AlCl_3$–HCl) 243, 266, 300sh, 334, and 410sh nm, $\lambda_{max.}$ (MeOH–NaOAc) 255sh, 264sh, 290sh, 347sh, and 390 nm. Under u.v. light a spot of the solution in methanol gave a brilliant fluorescent blue colour, changing to pale green on exposure to ammonia.

Quantitative Hydrolysis of Helichrysin.—A solution of helichrysin (20 mg) in hydrochloric acid (5%; 50 ml) was heated on a water-bath for 2 h. T.l.c. showed hydrolysis to be complete. The solution was cooled, extracted with ether and ethyl acetate to remove aglycones, neutralised, concentrated, and titrated against a standardised Fehling's solution. The precipitated copper(I) oxide was equivalent to 6.84 mg of glucose (Found: 34.2% glucose. $C_{22}H_{24}O_{10}$,-$2H_2O$ requires 37.3% for 1 mol. equiv. of glucose).

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