

A high content (—)-deguelin concentrate from a commercial derris resin by steady-state distribution

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The isolation of a high content (—)-deguelin concentrate from a commercial derris resin using steady-state distribution is described. The separation was followed by noting the change in ratio of extinctions at two wavelengths.

OF the six rotenoids occurring in *Derris* sp. only optically active deguelin is not known in a pure or crystalline form, although the crystalline (\pm) form has been obtained by treating a crude optically active deguelin concentrate with alkali (Haller & La Forge, 1934). An early claim to have isolated optically active crystalline deguelin from the resin (Takei, Miyajima & Ono, 1933) was later attributed to isolation of (\pm)-deguelin containing a small amount of rotenone responsible for the optical activity (Haller & La Forge, 1934).

We describe the isolation from a commercial derris resin of a high content (—)-deguelin concentrate which, although not crystalline was homogeneous and superior in deguelin content to that of Haller & La Forge (1934).

Experimental

The proton nmr spectra were determined at 60 megacycles on a Varian A-60 spectrometer with tetramethylsilane as internal standard. The light petroleum used had b.p. 60-80°.

STEADY-STATE DISTRIBUTION OF A COMMERCIAL DERRIS RESIN

A commercial derris resin (1.25 g), from which the rotenone had been removed as its carbon tetrachloride solvate, was dissolved in benzene (25 ml) and an aliquot (0.5 ml) of this solution was placed in cell (O) of a Quickfit and Quartz Steady-State Distribution apparatus. Distribution was made using benzene-light petroleum/acetic acid-water (6:14, 13:7) mixtures as the two phases by alternate top (T; upper phase) and bottom (B; lower phase) transfers. Aliquots (0.5 ml) of the resin solution were injected into cell (O) after each transfer and the distribution continued until 25 fractions of each phase had been collected from the ends of the train. Aliquots (0.05-0.5 ml) from the upper and lower phases were separately evaporated with a stream of nitrogen and the residues dissolved in spectroscopic ethanol (95%, 10 ml). The extinctions of the ethanolic solutions were determined at 237 and 272 $m\mu$ using a Uvispek H700 spectrophotometer.

The resin used had $E(1\%, 1\text{ cm})$ 529 and 356 at λ_{max} 237 $m\mu$ and λ_{max} 272 $m\mu$ respectively. The amount of material (mg) in each fraction from the distribution was calculated at each wavelength from the equation, $100 \times a \times b/c$, where a = dilution factor for aliquot taken, b = extinction of the aliquot in 10 ml of spectroscopic ethanol and c = $E(1\%, 1\text{ cm})$

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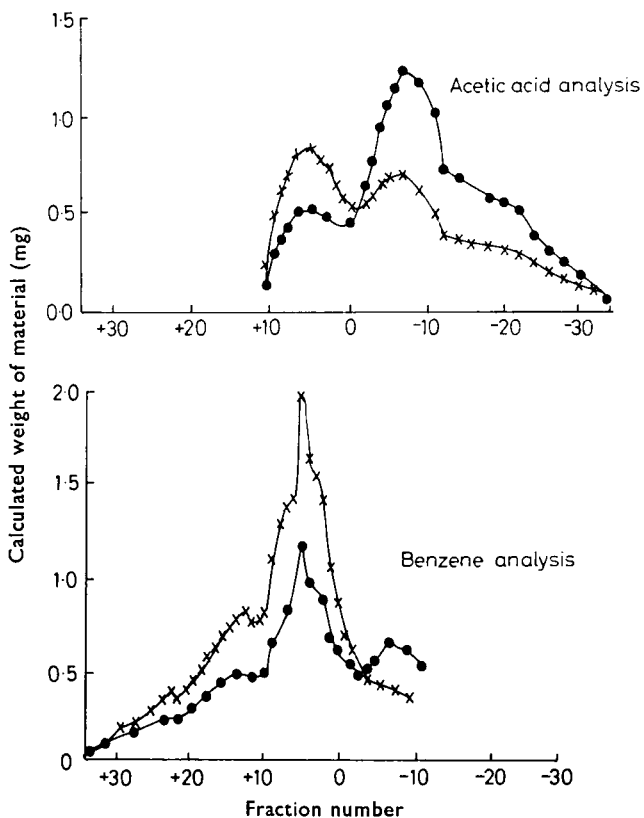


FIG. 1. Steady-state distribution of derris resin using benzene–light petroleum/acetic acid–water solvent mixture (●—based on D at 237 $m\mu$, ×—based on D at 272 $m\mu$).

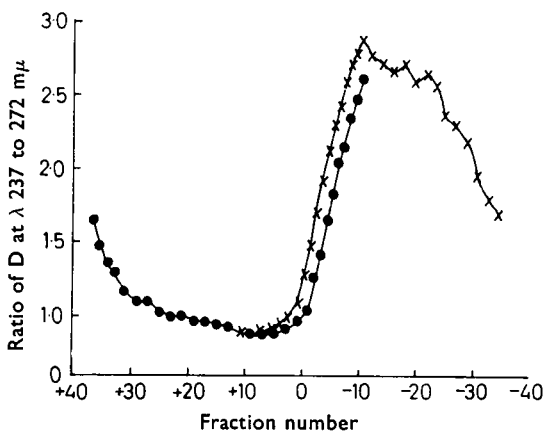


FIG. 2. Variation in ratio of D at λ 237 to 272 $m\mu$ with fraction number. (×—acetic acid analysis; ●—benzene analysis).

for the resin. The results of the analysis are shown in Fig. 1. The true fraction weight for absorbable material lies between the weights calculated at the two wavelengths assuming that absorbable materials have similar molecular weights. A graph of the ratio of extinctions at λ 237 and 272 $m\mu$ for the fractions shows a separation into high and low ratio fractions (Fig. 2).

Using this method, resin (11.1 g) was distributed between the same solvent mixture but with 16 cycles of the sequence TBBB. The fractions which had D 237/272 ratio < 1 were bulked and separated. The lower phase was diluted with water, extracted with ether and the organic phase neutralized (NaHCO_3), washed, dried (Na_2SO_4) and evaporated. The upper phase was neutralized, washed, dried and evaporated. The pooled residues (3.36 g) were dissolved in the minimum amount of benzene and chromatographed on a column of neutral alumina (Grade III) using ether-light petroleum (1:1) solvent mixture. The eluate was collected in five fractions (25 ml). Fractions (2) and (3), which contained the main bulk of the material, deposited crude dehydrorotenone crystals (44 mg) on standing. After removal of the crystals, the mother liquor was evaporated to give a white residue (2.5 g) which when dissolved in the minimum amount of carbon tetrachloride deposited impure rotenone-carbon tetrachloride solvate (0.56 g). The mother liquors were evaporated to give a non-crystallizable residue (1.55 g), m.p. 70° (glass) which gave positive reactions in the Goodhue (1936), Durham, and Rogers-Calamari (1936) tests but no colour with ferric chloride. Chromatography of the residue (0.32 g) on alumina with chloroform-light petroleum (1:1) solvent mixture with collection of the main fraction followed by removal of solvent left a crisp brown deguelin concentrate (0.3 g). The concentrate had a Goodhue value of 68.2%, $[\alpha]_{20}^{D} - 110.5^\circ$ (c 1%; benzene), λ_{max} [log $E(1\%, 1 \text{ cm})$], 237 (2.667), 250 (2.651), 271 (2.735), 295 (2.367) and 315 (i) (2.197) $m\mu$, ν_{max} (CHCl_3), 1667 (C=O), 1592, 1573, 1368, 1337, 1110, 1089, 909 and 893 cm^{-1} , and the proton nmr spectrum showed signals at τ 6.18, 6.21 (CH_3O -), 3.17, 3.52 (ring A, aromatic protons), 8.54, 8.61 ($6'-(\text{CH}_3)_2$ -), doublets at 2.22 ($J = 9$ cycles/sec, 11 H), and 3.51 ($J = 9$ cycles/sec, 10 H) and doublets at 3.30 ($J = 9.5$ cycles/sec, 4'H) and 4.42 ppm ($J = 9.5$ cycles/sec, 5'H).

CONVERSION OF DEGUELIN CONCENTRATE TO (\pm)-DEGUELIN WITH ALKALI

The method of Haller & La Forge (1934) in our hands gave appreciable quantities of tephrosin and was therefore modified. The concentrate (1.5 g; 44% Goodhue value) in methanol (75 ml) under an atmosphere of hydrogen was mixed with sodium hydroxide solution (0.6 ml, 2N). After 15 min the solution was neutralized with mineral acid and allowed to stand overnight. On cropping the solution, crystals (413 mg) were deposited which gave a single spot (Rf 0.6) on thin film chromatography on alumina, which fluoresced yellow with ultraviolet light and stained yellow when sprayed with solutions of either iodine or 2,4-dinitrophenylhydrazine. Recrystallization (180 mg) from methanol gave (\pm)-deguelin (120 mg), m.p. $170\text{--}171^\circ$ [lit. cites 165° (Cahn, Phipers & Boam, 1938) and 171°

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(Clark, 1931)] which had a Goodhue value of 83.6% (Goodhue & Haller, 1939, cite 81%). (Found: C, 70.0; H, 5.7. Calc. for $C_{23}H_{22}O_6$: C, 70.0; H, 5.6%).

OPTICAL ROTATION—RACEMIZATION STUDIES ON ROTENONE AND A DEGUELIN CONCENTRATE

The optical rotation of a solution of a deguelin concentrate (1%) in "Analar" benzene (10 ml) was measured using a 1 dm cell at 20°. A solution (0.75 ml) of methanolic potassium hydroxide (0.34 N) was then added and the optical rotation noted at intervals. The $[\alpha]_{20}^D$ declined from an initial reading of -78° to zero within 10 min. Under similar conditions, solutions of rotenone (1, 1.5, 2.0%) which gave initial $[\alpha]_{20}^D$ values of -216° , -208° and -217° respectively declined to a final value of -33° , -44° and -49° respectively.

HIGH RATIO FRACTION

Fractions from the steady-state distribution of the crude resin with a ratio > 2 were bulked, the upper and low phases separated and processed as previously described. The resinous material (1.11 g) from the lower phase was chromatographed on alumina using benzene-chloroform (1:1) solvent mixture. The green ultraviolet fluorescent eluate was collected in two fractions, which were then evaporated to give residues (1) 0.57 g, (2) 0.30 g. Residue (1) alone gave a positive Durham reaction and had a Goodhue value of 43%. This residue (200 mg) was dissolved in methanol and kept at 0°. An oily mass was deposited which on standing at room temperature (20°) gave crystals (40 mg) of (–)-elliptone, which had m.p. and mixed m.p. 170–171.5° and 173° respectively (with an authentic sample of (–)-elliptone m.p. 174.5–175°), λ_{\max} (log ϵ) 238.7 (4.575) and 274.7 m μ (3.949) (–)-elliptone had λ_{\max} 238.7 (4.616) and 274.7 m μ (3.959).

Discussion

The main constituent of the low ratio fraction was recognized as deguelin by its proton nmr spectrum which was similar to that described for (\pm)-deguelin (Crombie & Lown, 1962) and is readily distinguishable from other known non-phenolic rotenoids by the signals at τ 3.3 and 4.42 ppm (doublets, $J = 9.5$ cycles/sec) corresponding to the two ring E olefinic protons. The infrared and ultraviolet absorption spectra were in accord with this interpretation which was confirmed by the isolation of (\pm)-deguelin from the concentrate after treatment with alkali.

The homogeneity of the deguelin concentrate was investigated by thin-layer chromatography and optical rotation-racemization studies. Thin layer chromatography on alumina gave a single spot (Rf 0.6) showing that tephrosin (Rf 0.3), known to be formed by oxidation of deguelin on the column (Brierley, 1966; Rangaswani & Sastry, 1956), was absent. In the optical rotation-racemization studies, rotenone had a final average specific rotation value of -42° , corresponding to the formation of mutarotenone

by racemization of the C-6a, C-12a centres whereas the deguelin concentrate had a final rotation of zero. It is concluded that the optical activity of the concentrate is due to (–)-deguelin and that rotenone is absent within the limits of sensitivity of the method used.

The (–)-deguelin concentrate isolated in this work although it could not be obtained crystalline, was apparently homogeneous as judged by the techniques employed and contained at least 85% deguelin as shown by colorimetric assay. In these respects it is superior to the concentrate previously described by Haller & La Forge (1934) which contained about 50% deguelin together with an appreciable quantity of rotenone.

The ultraviolet absorption spectrum for the high ratio fraction obtained in this work suggested a similarity to several recently isolated flavanoids from other *Leguminosae* sp. (nepseudin, Crombie & Whiting, 1962; neotenone, Crombie & Whiting, 1963) and differed considerably from that quoted for (–)-elliptone by Meijer (1941). However, the presence of (–)-elliptone in the fraction was indicated by the proton nmr spectrum and confirmed by isolation of crystalline (–)-elliptone.

The method described here for location of the high and low ratio fractions in the counter-current train is rapid for locating the constituents of a mixture, and is particularly advantageous where incomplete separation has occurred. The usual tedious gravimetric procedure is avoided and furthermore the constituents can be located in instances where a weight analysis shows little separation (cf. Figs 1 and 2). However, for its successful application the constituents must have different ratios for their ultraviolet absorption extinction coefficients at two pre-selected wavelengths. The method described here could prove useful in detecting and following the isolation of impurities in drugs where steady state distribution or column chromatography is being used.

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