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# THE STRUCTURE AND STEREOCHEMISTRY OF TWO NEW DIHYDROFLAVONOL GLYCOSIDES

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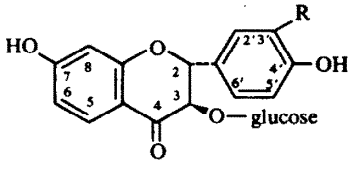
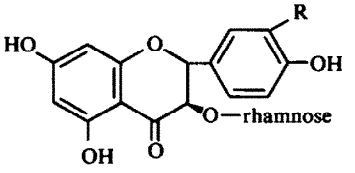
**Abstract**—Lecontin and (+)-fustin glucoside, two new members of the rare group of flavonoids the dihydroflavonol 3-O-glycosides, are shown to have structures I and II, respectively. ORD, CD and NMR data are presented for these and other dihydroflavonol 3-O-glycosides and the relationship between absolute stereochemistry and the order and sign of Cotton effects in this class of glycosides is established.

METHANOL–water extraction of *Baptisia lecontei* (family Leguminosae) leaf and stem material yielded two new dihydroflavonol glycosides, lecontin (I) and (+)-fustin 3-O-β-D-glucoside (II). These compounds are members of a rare group of natural products, the 3-O-glycosylated dihydroflavonols, which at present is represented only by two compounds, engelitin (III) and astilbin (IV). This is the first report of the biogenetically unusual 5-deoxy derivatives.




I: R = H
II: R = OH




III: R = H
IV: R = OH

Lecontin (I) was isolated in 0·01% yield as a paper chromatographically pure glass. A dihydroflavonol constitution for lecontin was indicated by the UV data [$\lambda_{max}$ (MeOH) 230, 277, 310 (sh) nm; log ε, 4·21, 4·11, 3·85] and also by the acid and β-glucosidase hydrolyses which produced 4',7-dihydroxyflavonol and 4',7-dihydroxydihydroflavonol, respectively.

The 60 Mc NMR spectrum of lecontin trimethylsilyl (TMS) ether (Fig. 1) was consistent with a 3,4',7-oxygenation pattern when compared with that of engelitin (Fig. 2), a dihydroflavonol glycoside of known structure.[1] Lecontin, lacking the 5-OH group present in engelitin, showed a typical low field C-5 proton signal at 7·75 ppm ($J$ = 9 c/s), and the coupling of the C-5 proton with the C-6 proton accounts for the major differences between the two NMR spectra. In the spectrum of lecontin TMS-ether the H-2 and H-3 protons were present at 5·40 and 4·60 ppm, respectively, and the coupling constant of 11 c/s is that expected for *trans*-diaxial protons.[2]

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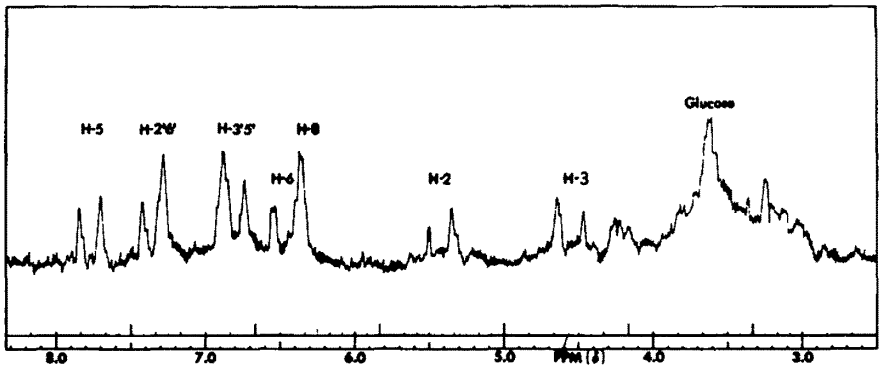



FIG. 1. NMR Spectrum of trimethylsilylated lecontin (I).

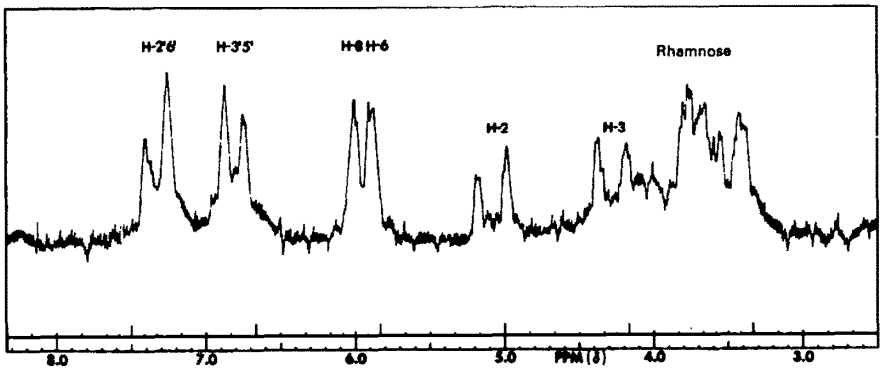



FIG. 2. NMR Spectrum of trimethylsilylated engelitin (III).

Signals between 3·0 and 4·3 ppm in the NMR spectrum of lecontin TMS-ether suggested that lecontin was a monoglycoside. The sugar was subsequently identified as D-glucose by gas chromatography of its TMS-ether and a β-linkage of the glucose to the aglycone was indicated by the hydrolysis of lecontin with β-glucosidase. The position of attachment of the D-glucose was established as the 3-OH by complete methylation of the glycoside followed by acid hydrolysis; the resultant aglycone showed the physical[3] and spectral properties expected for 4',7-dimethoxyflavonol.

(+)-Fustin 3-O-β-D-glucoside (II), like lecontin, was isolated in low yield (0·0003%) as a paper chromatographically pure glass. The UV spectrum of II [$\lambda_{max}$ (MeOH) 232, 279, 310 nm] was almost identical with that of dihydrofisetin ($\lambda_{max}$ 235, 279, 310 nm; log ε, 4·19, 4·17, 3·84), and a 3,7,3',4'-oxygenation pattern was confirmed by acid hydrolysis, which produced fisetin quantitatively. Acid hydrolysis of II also yielded glucose, which was identified by paper chromatography, and by using the quantitative procedure of Pridham[4] the sugar:aglycone ratio was established as 1:1. The position of attachment of the glucose moiety was shown to be the 3-OH group by comparing the effect of added $AlCl_3$ on the UV spectra of both II and its aglycone, dihydrofisetin (VI). With added $AlCl_3$, the dihydrofisetin spectrum changed

drastically [$\lambda_{max}$ (MeOH/$AlCl_3$) 235, 303, 340 (sh) nm] due to the $AlCl_3$ complex formation between the C-4 ketone and the free 3-OH group, but the fustin glycoside, lacking a free 3-OH group, showed no change. Hydrolysis of this fustin 3-O-glucoside to fisetin with β-glucosidase indicated that the glucose was β-linked.

The absolute configurations at C-2 and C-3 in both I and II were deduced from ORD and CD studies. Previous ORD and CD work on naturally occurring dihydroflavonol aglycones[5–7] is limited in scope. A positive Cotton effect has been associated with the 2*R*:3*R* configuration in leaserone,[6] and a published ORD curve for the trimethyl ether of (+)-fustin[7] shows positive extrema at 224, 280 and 338 nm and a negative extremum at 312 nm. No data however appear to be available on the ORD of dihydroflavonol glycosides, except for the narrow range (400–500 nm) positive ORD curves published for engelitin (III) and astilbin (IV).[8]

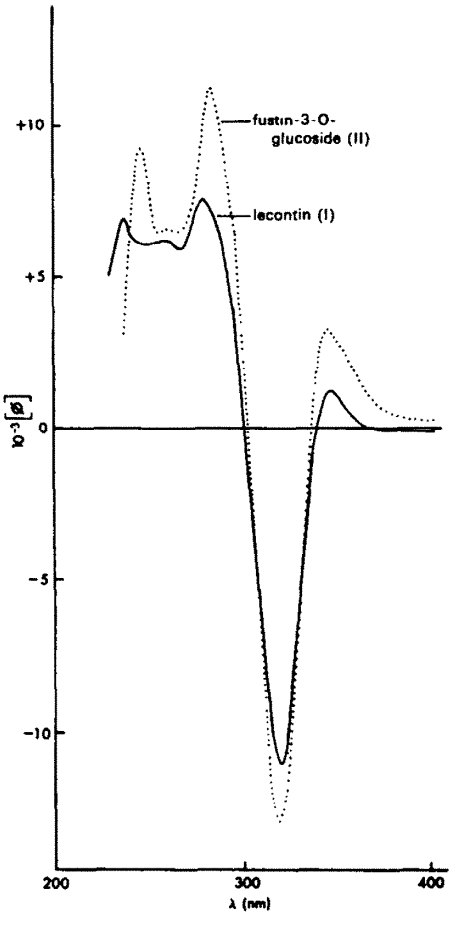



FIG. 3. ORD Spectra.

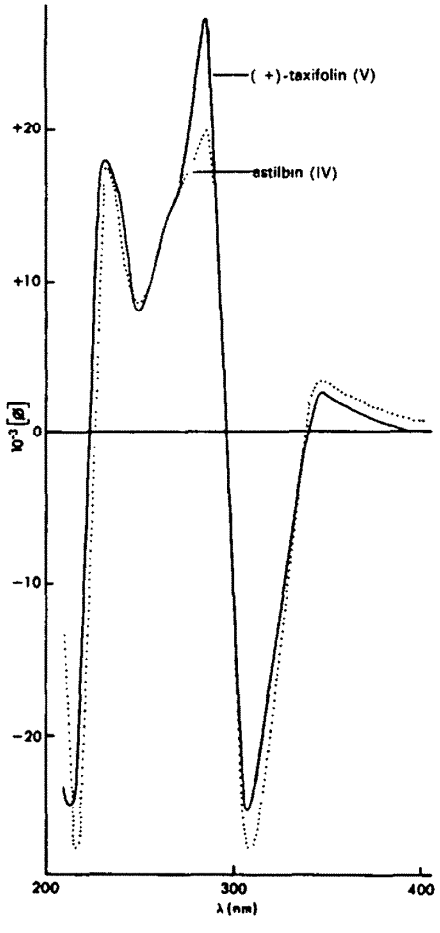
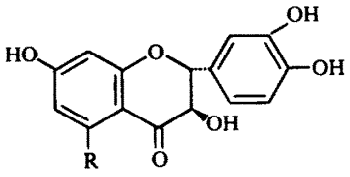



FIG. 4. ORD Spectra.

The ORD spectra of I and II (Fig. 3) are almost identical in sign and form and so require that the two natural products have the same absolute stereochemistry at C-2 and C-3.

The absolute stereochemistry of both I and II was established by comparing their ORD curves with those of (+)-taxifolin ($[\alpha]_D^{25} = +44°$; $c = 1{\cdot}03$, acetone:water, 1:1), engelitin (III) and astilbin (IV), all of which possess *trans*-diaxally related C-2 and C-3 protons (NMR evidence). The absolute stereochemistry of (+)-taxifolin (V) has been unequivocally determined as 2*R*:3*R*,[9] and its ORD curve (Fig. 4) shows the same general form as those of lecontin (I), fustin 3-O-glucoside (II) and the recently published (+)-fustin trimethyl ether.[7] Thus, the absolute stereochemistry of I and II is defined as 2*R*:3*R* if the sugar residue at C-3 does not cause curve inversion. No




V: R = OH
VI: R = H

inversion or change of form, however, is noted in the ORD curve of (+)-taxifolin 3-O-L-rhamnoside (astilbin, Fig. 4), and an ORD curve of crude lecontin aglycone showed the same general form as did lecontin. Since glycosylation at the 3-OH group in these dihydroflavonols has little effect on the ORD curves, lecontin and (+)-fustin 3-O-β-D-glucoside can be assigned structures I and II.

In order to determine the number and sign of Cotton effects represented by the ORD curves, CD curves were measured for astilbin and (+)-taxifolin. Four Cotton effects in the range 220–400 nm were observed for both astilbin [$\lambda_{max}$ (dioxan) < 230, 250–255, 293, 333 nm; $\Delta\varepsilon > +8$, $+3{\cdot}3$, $-11{\cdot}9$, $+4{\cdot}9$] and (+)-taxifolin [$\lambda_{max}$ (dioxan) < 235, 260–275, 295, 330 nm; $\Delta\varepsilon > +4{\cdot}0$, $+2{\cdot}5$, $-10{\cdot}6$, $+2{\cdot}65$]. The above data suggest therefore that all simple polyoxygenated dihydroflavonols (and their 3-O-glycosides) that possess the *trans*-2*R*:3*R* absolute configuration will give CD and ORD curves which show four Cotton effects in the order (+), (−), (+), (+) from 400 to 220 nm.

## EXPERIMENTAL

NMR spectra were determined using a Varian A-60 spectrometer using $CCl_4$ as solvent and TMS as internal standard; the ORD spectra were measured in MeOH on a Cary model 60 spectrometer. All relevant NMR, ORD and CD data are presented in the text and not in the experimental. M.ps are uncorrected. All two-dimensional paper chromatography was carried out on Whatman 3MM paper at 25° using t-BuOH:HOAc:$H_2O$, 3:1:1 and 15% aqueous HOAc. The solvent systems are hereafter referred to as TBA and HOAc, respectively.

*Lecontin* (I). Dry leaf and stem material of *B. lecontei* (2·8 kg) was extracted for 3 days with 15% aqueous MeOH (6·5 l.); yield: 470 g of extract. The same plant material on re-extraction with 50% aqueous MeOH (12 l.) yielded an additional 208 g of extractives. A portion of this second extract (23 g) was chromatographed on charcoal (75 g) in a Buchner funnel using successively as eluents: $H_2O$ (500 ml) MeOH (1500 ml), 7% aqueous phenol (1500 ml) and 25% methanolic phenol (500 ml). The aqueous phenol fraction yielded 3 g of phenol-free residue which was chromatographed 3 times on polyamide columns using as eluent, $H_2O$ containing increasing amounts of MeOH. Lecontin (19 mg) was obtained as a chromatographically pure clear glass from the 4:1 $H_2O$/MeOH fraction: m.p. 125–130°, $[\alpha]_D^{25} = -16{\cdot}75°$ ($c = 1{\cdot}83$, $H_2O$), $\lambda_{max}$ (MeOH) 230, 277, 310 (sh) nm (log ε, 4·21, 4·11, 3·85); $\lambda_{max}$ (NaOAc) 256, 338 nm; $\lambda_{max}$ ($NaOCH_3$)

250, 339 nm, $R_f$ (HOAc) 0·80; $R_f$ (TBA) 0·77. Complete NMR and ORD spectra are presented in Figs 1 and 3.

*Hydrolysis of lecontin* (I). Lecontin (2 mg) was hydrolysed with 5% HCl aq (3 ml) for 2 hr at 100°. The product was a yellow solid, m.p. 275°, $\lambda_{max}$ (MeOH) 257, 318, 357 nm, $R_f$ 0·74 (TBA), 0·0 (HOAc), which was identical in all respects with authentic 4',7-dihydroxyflavonol. When hydrolysed with β-glucosidase in distilled $H_2O$, lecontin gave a 1 : 1 mixture of 4',7-dihydroxyflavonol with another compound. This additional compound oxidized to 4',7-dihydroxyflavonol on prolonged exposure to air and co-chromatographed with authentic 4',7-dihydroxy-dihydroflavonol in both TBA and HOAc. When the acid hydrolysis was carried out under $N_2$, the 4',7-dihydroxy-dihydroflavonol was the only product.

The sugar was gas chromatographed as its trimethylsilyl ether derivative according to the procedure of Kagan and Mabry,[10] and showed retention times identical to those of an authentic glucose derivative.

*Methylation of lecontin* (I). The glycoside (I) in MeOH was treated with $CH_2N_2$ until the product gave no UV spectral shifts with the addition of NaOMe or $AlCl_3$. Subsequent acid hydrolysis of this methylated product yielded a yellow oil which sublimed at 180° and 0·05 mm to give a yellow solid, m.p. 185–190° (4',7-dimethoxyflavonol has m.p. 193°),[3] $R_f$ 0·10 (HOAc), 0·83 (TBA); $\lambda_{max}$ (MeOH) 258, 275 (sh), 314, 348 nm; $\lambda_{max}$ ($AlCl_3/H^+$) 271, 322 (sh), 410 nm.

(+)-*Fustin* 3-O-β-D-*glucoside* (II). A portion (66 g) of the 50% aqueous MeOH extract of *B. lecontei* (see lecontin isolation) was separated into water soluble and water insoluble fractions. Material obtained from the former (48 g) was extracted with hot MeOH and the soluble portion (31 g) was chromatographed twice on "polyclar" (polyvinylpyrrolidone) in MeOH/$H_2O$ to give other flavonoids. A final elution of the second column with a mixture of MeOH (200 ml) and 3N HCl (3 ml) yielded a small quantity of oil containing fustin glucoside (II). 2D Paper chromatography of this oil in TBA and HOAc separated II as a pale blue fluorescent spot (in UV light), $R_f$ 0·57 (TBA), 0·78 (HOAc). Elution of this spot from 10 chromatograms gave II as a colorless glass (1 mg); $\lambda_{max}$ (MeOH) 232, 279, 310 nm (log ε, 4·20, 4·18, 3·80), unchanged on addition of $AlCl_3$, $\lambda_{max}$ (NaOAc) 255, 286, 336 nm, $\lambda_{max}$ (NaOAc/$H_3BO_3$) 285, 317 nm. ORD data are presented in Fig. 3.

*Hydrolysis of* (+)-*fustin* 3-O-*glucoside* (II). Treatment of II with β-glucosidase in distilled water produced a compound which was detected by paper chromatography as a brilliant yellow fluorescent spot in UV light, $R_f$ 0·55 (TBA) and 0·03 (HOAc). This compound was spectrally and chromatographically identical with authentic fisetin.

The sugar was quantitatively determined by the method of Pridham.[4] Glycoside II (0·14 mg) was hydrolyzed with 6% HCl aq and the resultant free sugar (0·05 mg, 0·85 mole equivalents) had an $R_{glucose}$ value of 1·0 in EtAc:Py:$H_2O$, 12:5:4.

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