
ISOFLAVONES OF THE HEARTWOOD OF DALBERGIA RETUSA

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Abstract—Ether extracts of the heartwood Dalbergia retusa yield two crystalline isoflavones, identified by degradation, spectra, and synthesis as 7,8-dihydroxy-4'-methoxyisoflavone (retusin) and 7-hydroxy-8,4'-dimethoxyisoflavone (8-O-methylretusin).

INTRODUCTION

The heartwood of the Panamanian tree, Dalbergia retusa (cocobolo), is extremely resistant to attack by marine boring organisms.1 In addition to the quinone pigments which are described in a related paper, ether extracts of the heartwood yield major quantities of a colorless, crystalline phenol, C_{16}H_{12}O_{5}, m.p. 249°, now called retusin and identified as 7,8-dihydroxy-4'-methoxyisoflavone Ia, and minor amounts of a second colorless phenol,


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C_{17}H_{14}O_{5}, m.p. 221°, shown to be the 8-O-methyl derivative IIa of retusin. These isoflavones, which constitute approximately 1% of the weight of the heartwood, appear to be the first 7,8,4'-trioxygenated isoflavones reported from plant sources.

* Dalbergia* is in the Leguminosae, and a number of different isoflavones have been detected in other species of this genus. These include biochanin-A, genistein, caviunin, and 7-O-methyltectorigenin, which occur free or as O- or C-glycosides in various parts of *D. lanceolaria*,2 *D. sissoo*,3,4 *D. paniculata*5 and *D. nigra*.6,7 5-Deoxyisoflavones have also been detected in some species. These include formomononetin from the heartwood of *D. baroni*8 and *D. barretoana*,9 7,4'-dimethoxyisoflavone from *D. violaceae*,10 and $\psi$-baptigenin and formononetin, which co-occur with biochanin-A and caviunin in the heartwood of *D. spruceana*.10 *Machaerium villosum*, which is closely related to *Dalbergia*, contains daidzein, formononetin, isoflorone, 7,4'-dihydroxy-3'-methoxyisoflavone, and 7,3',4'-trihydroxyisoflavone.11 Oliveira *et al.*12 very recently reported that 5-deoxyisoflavones (structures unspecified) occur in five other *Dalbergia* species, viz. *D. obtusa, D. frutescens, D. cearensis, D. ecastophyllum* and *D. volubilis*.

**RESULTS**

Retusin contains one methoxyl and two phenolic hydroxyl groups. In accord with the proposed isoflavone structure it reacts slowly with Mg-HCl to give a red-brown solution, and its UV spectrum13 in ethanol shows a single $\lambda_{\text{max}}$ of high intensity at 261 nm ($E = 3.3 \times 10^{4}$) with only an inflection of low intensity at 308 nm ($E = 6.53 \times 10^{3}$). Retusin forms a di-O-methyl derivative (m.p. 151°), which is hydrolyzed by alcoholic KOH to yield a deoxybenzoin, subsequently identified as IIIb.

Retusin rapidly reduces ammoniacal AgNO$_3$, gives a brilliant green color with ethanolic FeCl$_3$, and its $\lambda_{\text{max}}$ undergoes an 8 nm bathochromic shift on addition of boric acid-sodium acetate.13 Oxidation of retusin with conc HNO$_3$ yields traces of a compound, which, on the basis of its m.m.p., appears to be 3-nitroanisic acid. These data indicate location of the methoxyl at the 4' position in the B ring, and an *ortho*-orientation of the two hydroxyls in the A ring. The presence of the *ortho*-dihydroxy grouping was chemically confirmed by the facile formation of a crystalline diphenylmethene derivative-IV, when retusin was heated briefly14 with a,a-dichlorodiphenylmethane.

In the presence of ethanolic sodium acetate the $\lambda_{\text{max}}$ of retusin undergoes a pronounced bathochromic shift (16 nm) to 277 nm, indicating13 location of one hydroxyl at position 7, and, therefore, the other at position 8, as in Ia, or at position 6, as in texasin V. Since the

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spectral and other reported properties of texasin\(^1\) (m.p. 285-287\(^\circ\)) and its di-O-methyl derivative\(^2\) (m.p. 174-175\(^\circ\)) distinctly differ from those of retusin, the latter can only be formulated as the 7,8-dihydroxyisoflavone derivative Ia. The NMR spectra of retusin derivatives fully confirm this structure. Thus, the 100 MHz spectrum of retusin diacetate (m.p. 166-167\(^\circ\)) in CDCl\(_3\) shows the protons at C\(_2\) as a singlet at 67.94, and the protons at positions 3' (5'), 2' (6'), and 6 as ortho-coupled doublets (\(J = 9.0\) Hz) at 86.96, 7.47 and 7.25, respectively. The proton at C\(_5\), ortho- to the carbonyl group, occurs well downfield as a doublet (\(J = 9.0\) Hz) at 88.20. The structure IIb was assigned to the deoxybenzoin formed by alkaline hydrolysis of di-O-methylretusin on the basis of, (a) its intense, wine-red ferric reaction, (b) its formation of a monomethyl derivative (m.p. 54\(^\circ\)), and (c) its 100 MHz spectrum in CDCl\(_3\). The latter showed the presence of the three methoxyls as 3H singlets at \(\delta 3.77, 3.87,\) and \(3.89,\) the benzylic methylene as a 2H singlet at \(\delta 4.15,\) and the protons at positions 6, 3' (5'), 2' (6'), and 5 as ortho-coupled doublets (\(J = 9.0\) Hz) at 86.47 (1H), 86.85 (2H), 87.18 (2H), and 87.61 (1H), respectively. The chelated hydroxyl appears downfield as a singlet at 812.55.

The structure of retusin was also confirmed synthetically. Thus, alkaline hydrolysis of IV, the \(\alpha,\alpha\)-diphenylmethylene derivative of retusin, gave a crystalline deoxybenzoin VI. Acid hydrolysis of VI then gave a crystalline trihydroxydeoxybenzoin, m.p. 157\(^\circ\). This trihydroxy compound was considered to be IIIa, although a substance of this structure had previously been synthesized by BF\(_3\) catalyzed condensation of 4-methoxyphenylacetic acid with pyrogallol and was reported\(^3\) to melt at 145-146\(^\circ\). Repetition of this synthesis gave a trihydroxydeoxybenzoin, m.p. 157\(^\circ\), identical in all respects with the product from retusin. The structure of the synthetic product as IIIa was established by its formation of a di-O-methyl derivative, identical with the deoxybenzoin IIIb from di-O-methyl retusin, and by its formation of a triacetate (m.p. 126\(^\circ\)), whose NMR spectrum showed the presence of three acetyl groups, a benzylic methylene group, and six ortho-coupled protons. Reaction of synthetic IIIa with ethyl orthoformate\(^7\) gave 7,8-dihydroxy-4'-methoxyisoflavone, which was identical with the natural product.

The second, minor phenol, m.p. 221\(^\circ\), from *Dalbergia retusa* contains two methoxyl and one hydroxyl groups, and on methylation it gives di-O-methyl retusin Ib. The \(\lambda_{\text{max}}\) of this new isoflavone in ethanol (256 nm) shifted to 270 nm on the addition of sodium acetate, indicating the location of the free hydroxyl at position 7 as in structure IIa. The 100 MHz NMR spectrum of the acetate (m.p. 124\(^\circ\)) of this isoflavone showed the proton at C\(_6\) as an ortho-coupled doublet at 87.11, and an upfield shift (relative to retusin diacetate) of the C\(_5\) proton doublet to 88.04. The structure of this isoflavone as 8-O-methylretusin was confirmed by its synthesis from 7,8-diacetoxy-4'-methoxyisoflavone Ic. Selective benzylation\(^8\) of Ic and alkaline hydrolysis of the product gave 7-benzyloxy-8-hydroxy-4'-methoxyisoflavone VIIa. This was methylated and then catalytically hydrogenolyzed to yield 7-hydroxy-8,4'-dimethoxy-isoflavone IIa, identical in all respects with the natural product.

Further confirmation of the structure of the above isoflavone was provided by the synthesis of the isomeric 8-hydroxy-7,4'-dimethoxy-isoflavone VIIIb, by (a) selective methylation of 7,8-diacetoxy-4'-methoxyisoflavone and (b) in good yield by reaction of the deoxybenzoin VIII with POCl\(_3\) and dimethylformamide. The properties of VIIIb (m.p.

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203–204°C; diacetate, m.p. 154–155°) differ markedly from those of the natural isoflavone IIa, and its λ<sub>max</sub> in ethanol (260 nm) does not shift in the presence of sodium acetate.

**EXPERIMENTAL**

Isolation of retusin Ia and 8-O-methyl retusin Ila. Sawdust of Dalbergia retusa heartwood (2500 g) was extracted continuously with low boiling petroleum for 2 days, and then with ether for 2 days. The residue (253 g) obtained on evaporation of the ether extract was heated with benzene (2 x 500 ml) and the undissolved, cream-colored crystalline residue was collected (25.0 g). TLC on silicic acid showed that this residue consisted chiefly of retusin, contaminated with a small quantity of 8-O-methylretusin. The residue was dissolved in warm acetone (1 l), concentrated to 300 ml, diluted with EtOAc (500 ml) and reconstituted to 600 ml. On cooling, crude retusin separated as cream-colored crystals (m.p. 243-245°C) (16.0 g). Concentration of the EtOAc filtrate yielded further quantities of retusin (5.7 g). The EtOAc filtrate was then passed through a short column of silicic acid impregnated with boric acid, Evaporation of the filtrate gave chromatographically homogenous 8-O-methylretusin (1.07 g). 8-O-Methylretusin was also obtained from the petroleum extract of the wood. The orange solid which separated from the extract was heated with ether (250 ml) and filtered. The ether extract was evaporated and the residue was dissolved in a minimal volume of hot benzene. Recrystallized from acetone-MeOH and from acetone alone retusin Ia separated as colorless, glistening prisms, m.p. 157°C, undepressed with optic rotation (0.52 g) which gave an intense wine-red color with alcoholic FeCl₃ (Found: C, 67.6; H, 6.01; MeO⁻, 30.6. Calc. for C₁₇H₁₈O₅: C, 67.5; H, 6.00; 3 MeO⁻, 30.8). Methylation of IIb gave 4-methoxybenzyl 2,3,4-trihydroxyphenyl ketone, glistening, brittle needles from petroleum, m.p. 157°C (Found: C, 70.5; 1H, S, 67.92, J = 9.0 Hz; 2H, d, 66.21, J = 9.0 Hz; 2H, d, 66.23, J = 9.0 Hz. Ethylation of retusin gave di-O-ethyl-retusin from MeOH as colorless, soft needles, m.p. 110°C (Found: C, 70.5; H, 5.92. Calc. for C₂₀H₂₀O₅: C, 70.6; H, 5.92). 100 MHz NMR spectrum in CDCl₃: 3H, t, 5.144, J = 7.0 Hz; 3H, S, 5.144, J = 7.0 Hz; 3H, S, 5.144, J = 7.0 Hz. Ethylation of retusin diacetate with Me₂SO₄-K₂CO₃-Me₂CO for 2 hr gave a solid which was crystallized from acetone-MeOH to give IV as glistening, slightly brown plates, m.p. 146°C (0.81 g). With alcoholic FeCl₃ VI gives an intense red color (Found: C, 76.7; H, 5.10. Calc. for C₁₉H₁₈O₅: C, 76.7; H, 5.06). 100 MHz spectrum in CDCl₃: 2H, d, 67.52, J = 9.0 Hz; 2H, d, 67.68, J = 9.0 Hz; 2H, d, 67.84, J = 9.0 Hz; 2H, d, 67.16, J = 9.0 Hz; 2H, d, 67.50, J = 9.0 Hz; 2H, d, 67.56, J = 9.0 Hz; 2H, d, 67.60, J = 9.0 Hz; 2H, d, 67.54, J = 9.0 Hz. 4-Methoxy-7,8-diphenylmethyleneoxysaflavone IV was obtained as glistening, slightly brown colored, needles, m.p. 198°C (Found: C, 77-6; H, 4.53. Calc. for C₁₉H₁₈O₅: C, 77-7; H, 4.50).

4-Methoxybenzyl 2-hydroxy-3,4-diphenylmethyleneoxysaflavone VI. A solution of IV (1.0 g) in EtOH (80 ml) and 10%aq. KOH (80 ml) for 1 hr. The product was recrystallized from acetone-MeOH to yield 4-methoxybenzyl 2-hydroxy-3,4-dimethoxyphenyl ketone IIIb as thick, colorless needles, m.p. 151°C (1.8 g) (Found: C, 69.4; H, 5.09; MeO⁻, 29-7. Calc. for C₁₅H₁₄O₅: C, 69.2; H, 5.16; 3 MeO⁻, 29.8). 100 MHz NMR spectrum in CDCl₃: 3H, 5.83, J = 12.4 Hz; 6H, S, 64.00; 1H, d, 66.95, J = 9.0 Hz; 2H, d, 87.05, J = 9.0 Hz; 1H, S, 67.98; 1H, d, 88.03, J = 9.0 Hz. Ethylation of retusin gave di-O-ethyl-retusin from MeOH as colorless, soft needles, m.p. 110°C (Found: C, 70-5; H, 5.92. Calc. for C₁₅H₁₄O₅: C, 70.6; H, 5.92). 100 MHz spectrum in CDCl₃: 3H, t, 5.144, J = 7.0 Hz; 3H, S, 5.144, J = 7.0 Hz; 3H, S, 5.144, J = 7.0 Hz. Ethylation of retusin diacetate with Me₂SO₄-K₂CO₃-Me₂CO for 2 hr gave a solid which was crystallized from acetone-MeOH to yield IV as colorless, glistening needles, m.p. 122-123°C (0.52 g), which gave an intense wine-red color with alcoholic FeCl₃ (Found: C, 67.6; H, 6.01; MeO⁻, 30.6. Calc. for C₁₅H₁₄O₅: C, 67.5; H, 6.00; 3 MeO⁻, 30.8). Methylation of IIIb gave 4-methoxybenzyl 2,3,4-trihydroxyphenyl ketone, glistening, brittle needles from petroleum, m.p. 151°C which did not give a color with alcoholic FeCl₃ (Found: C, 68.2; H, 6.46. Calc. for C₁₅H₁₄O₅: C, 68.3; H, 6.37). 100 MHz spectrum in CDCl₃: 3H, t, 5.144, J = 7.0 Hz; 3H, t, 5.144, J = 7.0 Hz; 3H, S, 63.82; 4H, t, 63.23, J = 7.0 Hz; 1H, d, 86.94, J = 9.0 Hz. Ethylation of retusin diacetate with Me₂SO₄-K₂CO₃-Me₂CO for 2 hr gave a solid which was crystallized from acetone-MeOH to give IIIb as colorless, glistening needles, m.p. 157°C (0.52 g) (Found: C, 69.4; H, 5.09; MeO⁻, 29-7. Calc. for C₁₅H₁₄O₅: C, 69.2; H, 5.16; 3 MeO⁻, 29.8).
The product crystallized from acetone-MeOH to give colorless needles, m.p. 160-163° (1.7 g). Mild alkaline were identical with those of di-0-methylretusin. 8-O-Methylretusin gave the colorless brittle prisms, m.p. 221°. It did not reduce AgNO₃ and it did not give a color with FeCl₃. (Found: C, 68.5; H, 4.75; MeO-, 20.9.) Acetylation gave 4'-acetox-7,8-diacetoxy-4'-methoxyisoflavone as colorless, brittle prisms, m.p. and m.m.p. with retusin, 248-249° (0.32 g). The synthetic isoflavone reduced AgNO₃, gave a brilliant green color with FeCl₃, and migrated as a single substance on silicic acid TLC when mixed with retusin (Found : C, 74.2; H, 5.31. Calc. for C₁₉H₁₆O₅: C, 74.2; H, 5.19.) 100 MHz spectrum in CDCl₃: 3H, s, 62.43; 3H, s, 63.85; 3H, s, 63.98; 2H, d, 87.26, J = 9.0 Hz. Methylation of synthetic IIa with CH₂N₂ in Et₂O and crystallization of the oily product from MeOH gave colorless needles, m.p. and m.m.p. with IIIb, 122°. Mixed with IIIb the product migrated as a single substance on silicic acid TLC (Rₖ 0.56 in Et₂O-petroleum, 2:1, 0.72 in benzene-EtOH, 9:1).

7,8-Dihydroxy-4'-methoxyisoflavone. Synthetic 4-methoxybenzyl-2,3,4-trihydroxyphenylketone IIIa (1.0 g) was heated under reflux with pyridine (2.0 ml), piperidine (4 drops), and ethyl orthoformate 17 (1.0 ml) for 7 hr. The product was collected and recrystallized successively from aq. MeOH and acetone-ETOAc to give 7,8-dihydroxy-4'-methoxyisoflavone as colorless, brittle prisms, m.p. and m.m.p. with retusin, 248-249° (0.32 g). The synthetic isoflavone reduced AgNO₃, gave a brilliant green color with FeCl₃, and migrated as a single substance on silicic acid TLC when mixed with retusin (Found : C 74.2; H, 5.31. Calc. for C₁₉H₁₆O₅: C 74.2; H, 5.19.) 100 MHz spectrum in CDCl₃: 3H, s, 62.43; 3H, s, 63.85; 3H, s, 63.98; 2H, d, 87.26, J = 9.0 Hz. Methylation of synthetic IIa with CH₂N₂ in Et₂O and crystallization of the oily product from MeOH (×3) gave colorless needles, m.p. and m.m.p. with IIIb, 122°. Mixed with IIIb the product migrated as a single substance on silicic acid TLC (Rₖ 0.56 in Et₂O-petroleum, 2:1, 0.72 in benzene-EtOH, 9:1).

7-Hydroxy-8,4'-dimethoxyisoflavone. A mixture of 7,8-diacetoxy-4'-methoxyisoflavone (1.1 g), PhCH₂Cl (2.1 ml), KI (1.0 g), K₂CO₃ (5 g) and dry acetone (15 ml) was heated under reflux for 16 hr. The product crystallized from acetone-MeOH to give colorless needles, m.p. 160-163° (1.7 g). Mild alkaline hydrolysis gave a solid which was crystallized successively from acetone-MeOH and from acetone alone. (Found : C, 68.5; H, 4.75; MeO-, 20.9.) Acetylation gave 8-O-methylretusin acetate which crystallized from MeOH as colorless needles, m.p. 135-136°, λₑₒₓ 256 nm. (Found : C, 74.2; H, 4.92. Calc. for C₂₅H₂₄O₄: C, 74.2; H, 4.92.)

7-Hydroxy-8,4'-dimethoxyisoflavone. VIIa. A mixture of 7,8-diacetoxy-4'-methoxyisoflavone (2.0 g), PhCH₂Cl (2.1 ml), KI (1.0 g), K₂CO₃ (5 g) and dry acetone (15 ml) was heated under reflux for 16 hr. The product crystallized from acetone-MeOH to give colorless needles, m.p. 160-163° (1.7 g). Mild alkaline hydrolysis gave a solid which was crystallized successively from acetone-MeOH and from acetone alone. (Found : C, 68.5; H, 4.75; MeO-, 20.9.) Acetylation gave 8-O-methylretusin acetate which crystallized from MeOH as colorless needles, m.p. 135-136°, λₑₒₓ 256 nm. (Found : C, 74.2; H, 4.92. Calc. for C₂₅H₂₄O₄: C, 74.2; H, 4.92.)

7-Hydroxy-8,4'-dimethoxyisoflavone. VIIa. 7-Benzyloxy-8,4'-dimethoxyisoflavone (0.70 g) was dissolved in MeOH (5 ml) and hydrogenated at atmospheric pressure over a 5 % W-C. 1 mol equiv. of hydrogen in benzene-EtOH, 9:1. 0.18 in benzene-EtOH, 9:1; 0.46 in Et₂O-petroleum, 2:1; 0.56 in Et₂O-petroleum, 2:1; 0.72 in benzene-EtOH, 9:1). (Found : C, 68.5; H, 4.75; MeO-, 20.9.) Acetylation gave 8-acetoxy-7,8-diacetoxy-4'-methoxyisoflavone. (Found : C, 68.5; H, 4.75; MeO-, 20.9.) Acetylation gave 8-acetoxy-7,8-diacetoxy-4'-methoxyisoflavone. (Found : C, 68.5; H, 4.75; MeO-, 20.9.) Acetylation gave 8-acetoxy-7,8-diacetoxy-4'-methoxyisoflavone.
$J = 9.0$ Hz; $1H, d, 87.09, J = 9.0$ Hz; $2H, d, 87.49, J = 9.0$ Hz; $1H, S, 87.91; 1H, d, 88.19, J = 9.0$ Hz.

(b) A solution of 3-methoxycatechol (6.0 g) and 4-methoxyphenyl-acetic acid (12.0 g) in ice-cold CHCl$_3$ (40.0 ml) was saturated with BF$_3$ and allowed to stand at room temp. for 2 days. H$_2$O (300 ml) and Et$_2$O (100 ml) were added and the crystalline solid was collected. The ether-CHCl$_3$ layer was evaporated and the residue was crystallized from aq. MeOH. This crystalline product was combined with the above crystalline product and recrystallized from acetone-MeOH. 4-Methoxybenzyl 2,3-dihydroxy-4-methoxyphenyl ketone VIII was thereby obtained as brittle, cream-colored prisms, m.p. 137° (12.0 g). (Found: C, 66.6; H, 5.61; MeO-, 21.6. Calc. for C$_{16}$H$_{14}$O$_5$: C, 66.7; H, 5.59; 2 MeO-, 21.5.) The diacetate of VIII crystallized from MeOH as colorless needles, m.p. 136–137°. (Found: C, 64.5; H, 5.49. Calc. for C$_{28}$H$_{26}$O$_{11}$: C, 64.5; H, 5.41.) VIII was converted into the isoflavone by adaptation of the method of Kagal et al.$^{19}$ VIII (2.88 g) was added to a solution of POCl$_3$ (1.8 ml) in N,N-dimethylformamide (5.0 ml) and the mixture was heated on a steam-bath for 1.5 hr. H$_2$O was added and the product was crystallized from acetone-MeOH. 8-Hydroxy-7,4'-dimethoxyisoflavone was obtained as colorless needles, m.p. and m.m.p. with product from (a), 203–204°. (1.6 g). The product formed a monoaacetate, m.p. and m.m.p. with the acetate of the product from (a), 154–155°.

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