

ISOLATION OF A NEW ISOMER OF (+)-LEUCOFISETINIDIN FROM *CASSIA MARGINATA* LEAVES*

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Cassia marginata Roxb. (family: Leguminosae) is a small deciduous tree growing in the forests from South Arcot to Travancore (India) and often planted for ornament.¹ The bark of the tree is a good tanning material and has been found to be a substitute for wattle and avaram.² The present communication describes the isolation and identification of (+)-catechin, butein 4'-glucoside (coreopsin), and a new dextrorotatory isomer of leucofisetinidin from the *Cassia marginata* leaves.

(+)-Catechin and butein 4'-glucoside were separated by cellulose column chromatography; the former was identified by comparison of its infrared spectrum, analytical values, and R_F values with those of the authentic sample and also by mixed melting points of the compound and its derivatives with authentic samples. Butein 4'-glucoside (coreopsin) was identified by thoroughly comparing it with an authentic sample. The presence of butein 4'-glucoside (coreopsin) was earlier noticed in several plants.^{3a} Though the compound seems to occur widely in nature, its presence in a Leguminosae plant is noted for the first time.

The (+)-leucofisetinidin was found to be a new dextrorotatory isomer of 7,3',4'-trihydroxyflavan-3,4-diol, since the melting points and rotation of this compound and its derivatives (see Experimental) were quite different from those of the corresponding isomers of (+)-7,3',4'-trihydroxyflavan-3,4-diol.^{3b,4,5}

Experimental

Two-dimensional paper chromatography was carried out using Whatman No. 1 paper in the solvent systems composed of (A) 6% acetic acid in the first way followed by (B) n-butanol-acetic acid-water (4 : 1 : 5, v/v) in the second way.⁶

Collection of the Plant Material

The leaves collected from a mature tree on the grounds of Central Leather Research Institute, Madras, were used in the experiments.

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¹ Gamble, J. S., "Flora of the Presidency of Madras." Vol. 1, p. 284. (Botanical Survey of India: Calcutta 1957.)

² Rao, J. B., and Nayudamma, Y., *Bull. cent. Leath. Res. Inst.*, 1955, **2**, 44.

³ Heilbron, I., "Dictionary of Organic Compounds." (a) Vol. 2, p. 738; (b) Vol. 4, p. 2624. (Eyre & Spottiswoode: London 1965.)

⁴ Rajadurai, S., *Leath. Sci.*, 1963, **10**, 340.

⁵ Rayudu, G. V. N., and Rajadurai, S., *Leath. Sci.*, 1965, **12**, 301.

⁶ Harborne, J. B., *J. Chromat.*, 1959, **2**, 581.

Preparation of Acetone Extract

Fresh leaves (2 kg) were crushed and 500 ml of water was added. After 1 hr, 4 l. of acetone was added; the mixture was kept at room temperature for 3 days. The acetone solution was decanted and a fresh lot of acetone was added; the process was continued till the extraction was complete. The combined acetone extract was then evaporated to 500 ml under vacuum (40°; nitrogen atmosphere) and filtered off from the chlorophyll and other viscous matter. The filtrate was evaporated to dryness in vacuum to give a reddish brown phenolic solid (50 g) which was successively extracted with ether (500 ml) to give fraction I (8 g) and ethyl acetate (300 ml) to give fraction II (10 g).

Separation of (+)-Catechin and Butein 4'-Glucoside from Fraction I

A glass column (90 by 3 cm) was filled with an aqueous suspension of Whatman ashless cellulose powder up to a length of 65 cm.

Fraction I (2 g) in methanol (5 ml) was placed on top of the column, which was then successively eluted with 6% acetic acid (250 ml; fraction A) and ethanol (200 ml; fraction B).

Fraction A on evaporation in vacuum (nitrogen) gave a solid, which on crystallization from water gave (+)-catechin (0.50 g), m.p. and mixed m.p. 176° (Found: C, 56.6; H, 5.5. Calc. for $C_{15}H_{14}O_6 \cdot 1.5 H_2O$: C, 56.8; H, 5.4%). $[\alpha]_D^{80} +17.4^\circ$ (c, 0.5 in acetone: water, 1:1), R_F 0.44 in A and 0.76 in B. The pentaacetate was prepared using acetic anhydride and pyridine and crystallized from ethanol, m.p. and mixed m.p. 130°.

Fraction B was evaporated to dryness in vacuum and the resulting solid (0.75 g) was taken up in water, warmed, and left overnight in the refrigerator. Butein 4'-glucoside separated as a yellow powder, m.p. and mixed m.p. 190° (Found: C, 58.2; H, 5.2. Calc. for $C_{21}H_{22}O_{10}$: C, 58.1; H, 5.1%). R_F 0.00 in A and 0.70 in B. The hexaacetate was crystallized from methanol, m.p. and mixed m.p. 184–186° (Found: C, 57.9; H, 5.3. Calc. for $C_{33}H_{34}O_{16}$: C, 57.7; H, 5.0%).

The acid hydrolysis of butein 4'-glucoside was carried out following the procedure of Shimokoriyama and Hattori.⁷ Butein, m.p. and mixed m.p. 212–214°, and glucose (osazone m.p. and mixed m.p. 204–206°) were found to be the only products of hydrolysis.

Isolation and Characterization of (+)-7,3',4'-Trihydroxyflavan-3,4-diol from Fraction II

Fraction II (4.0 g) was dissolved in ethyl acetate (25 ml) and dried (Na_2SO_4). Light petroleum (40–60°) was gradually added and the coloured sticky solid precipitated first was discarded. Further addition of light petroleum and cooling yielded the leucoanthocyanidin as a pinkish white amorphous powder. The process was repeated several times in order to obtain an almost colourless sample (1.5 g). The compound did not melt up to 300° (Found: C, 61.9; H, 4.9. Calc. for $C_{15}H_{14}O_6$: C, 62.1; H, 4.8%). $[\alpha]_D^{20} +3.79^\circ$ (c, 0.06 in acetone). R_F 0.61 in A and 0.57 in B. Sodium hydroxide degraded the compound into resorcinol (dibenzoate, m.p. and mixed m.p. 117°) and protocatechuic acid, m.p. and mixed m.p. 199°.

Conversion into the anthocyanidin chloride was effected by the method of Swain and Hillis⁸ and it had an absorption maximum in the visible range at 530 $m\mu$. The unidimensional ascending chromatography of the chloride was carried out on Whatman No. 3 filter paper using two solvent systems, viz., (i) Forestal solvent⁹ and (ii) 90% formic acid–3N hydrochloric acid (1:1, v/v).¹⁰ The R_F values obtained (0.75 and 0.43 respectively) were the same as those of the reference compound which was run under identical conditions.

The trimethyl ether was crystallized from ethyl acetate–light petroleum (40–60°), m.p. 150° (Found: C, 64.9; H, 6.2; OCH_3 , 28.1. Calc. for $C_{18}H_{20}O_6$: C, 65.1; H, 6.0; OCH_3 ,

⁷ Shimokoriyama, M., and Hattori, S., *J. Am. chem. Soc.*, 1953, **75**, 1900.

⁸ Swain, T., and Hillis, W. E., *J. Sci. Fd Agric.*, 1959, **10**, 63.

⁹ Roux, D. G., *Nature*, 1957, **179**, 305.

¹⁰ Geissman, T. A., "The Chemistry of Flavonoid Compounds." p. 76. (Pergamon Press: New York 1962.)

28.0%). $[\alpha]_D^{20} + 5.14^\circ$ (*c*, 0.07 in acetone). The diacetate was crystallized from ethyl acetate-light petroleum (40–60°), m.p. 167–169° (Found: C, 63.5; H, 6.2. Calc. for $C_{22}H_{24}O_8$: C, 63.5; H, 5.8%).

The pentaacetoxyflavan was prepared in the usual way and crystallized from ethyl acetate-light petroleum (40–60°), m.p. 172–174° (Found: C, 59.8; H, 5.0; $COCH_3$, 42.0. Calc. for $C_{25}H_{24}O_{11}$: C, 60.0; H, 4.8; $COCH_3$, 43.0%). $[\alpha]_D^{20} + 7.87^\circ$ (*c*, 0.1 in acetone).

Potassium Permanganate Oxidation of the Trimethyl Ether

Leucofisetinidin trimethyl ether (1.0 g) in acetone (100 ml) was refluxed with potassium permanganate (5.0 g) for 24 hr and the reaction mixture was worked up in the usual way; veratric acid (0.1 g), m.p. and mixed m.p. 181°, and 2-hydroxy-4-methoxybenzoic acid (0.05 g), m.p. and mixed m.p. 157°, were isolated and identified.

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