

Proanthocyanidins in the Bark of *Saraca asoca* Roxb. de Wilde

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Saraca asoca (Leguminosae), (–)-Epicatechin, Procyanidin (B2), 11'-Deoxyprocyanidin B

The bark of *Saraca asoca* is used as a main ingredient in the preparation of Asoka Arishta, a drug indigenous to Sri Lanka. In this paper an investigation on some biologically active constituents of the bark is described.

(–)-Epicatechin and procyanidin B2 were identified and a new compound, named 11'-deoxyprocyanidin B, was found. Its structure was elucidated by means of proton-NMR.

Introduction

Saraca asoca Roxb. de Wilde is well known for its medicinal use in India and Sri Lanka. According to an ayurvedic recipe [1], the astringent bark of this species, constitutes the main ingredient for the preparation of Asoka Arishta, a medicine used in Sri Lanka against menstrual disorders, like menorrhagia. A review on the ethnopharmacology of this drug has been published recently [2]. Subsequently we conducted experiments to find the possible mode of action of the drug. The experiments focused on influences on prostaglandin synthetase showed that some fractionated extracts containing procyanidins displayed remarkable inhibitory activities [3]. In this connection we carried out analytical work to identify the active constituents of the bark of *Saraca asoca*. The present communication deals with some of the active constituents of phenolic nature.

Results and Discussion

Several compounds have been described earlier to occur in the bark of *Saraca asoca*. Kaur Duggal and Misra [4] reported the presence of leucopelargonidin 3-O- β -D-Glucoside, leucopelargonidin and leucocyanidin in the stem bark. Behari *et al.* [5] isolated campesterol, stigmasterol and β -sitosterol, and Sen [6] noted the occurrence of haematoxin.

The bark material of *Saraca asoca* taken into our investigations was collected 1982 in Peradeniya, Sri

Lanka. A voucher specimen of parts of the species was deposited at the Rijksherbarium, Leiden, The Netherlands.

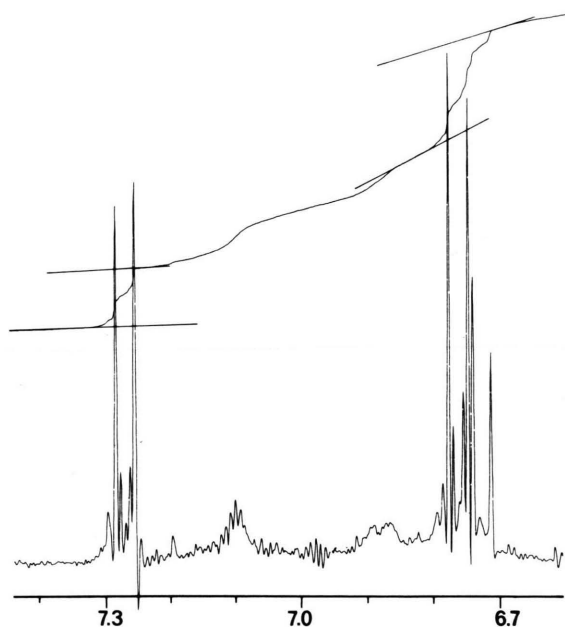
The powdered air dried bark was extracted successively with light petroleumether, diethylether, ethylacetate, methanol and water. The compounds under considerations here resulted from a chromatographic analysis of the ethylacetate extract. The first fractionation of this extract was performed on a polyamide column using methanol for the elution. Subsequently some fractions containing phenolics were collected and further purified on a Sephadex LH-20 column, again with methanol as eluting solvent. This resulted in the collection of three chromatographically pure compounds. The analysis of their molecular structure resulted in the identification of two known compounds, namely (–)-epicatechin and proanthocyanidin B2 [7]. The third single compound, termed compound C, has not been described before, and was proven to be a new proanthocyanidin. Its structure elucidation is described here.

Compound C: Treatment of compound C with 0.5 N hydrochloric acid at 60 °C for 15 min yielded a dark-red coloured solution. Chromatographic analysis of this coloured product on cellulose thin layer showed a single red spot which corresponds to a co-chromatographed authentic sample of cyanidin. Two-dimensional paper – as well as thin layer chromatography of compound C – showed R_f -values close to the known dimeric proanthocyanidin B2 [7]. In addition, its colour reactions with Fastblue Salt B (in neutral and alkaline solution), vanillin/H₂SO₄ and ferric chloride on thin layer, are very similar to those of proanthocyanidin B2 and the catechins. These data suggest a proanthocyanidin nature of the colourless to slightly yellow solid compound C. Its relationship with catechins and dimeric catechins is also expressed in its spectrophotometric characters. The absorption spectrum of compound C dissolved in methanol shows two wavelength maxima at 212 and 279 nm (proanthocyanidin B2 absorbs at $\lambda_{\max}$ 216 and 283 nm; epicatechin shows $\lambda_{\max}$ at 207 and 281 nm). Upon adding a drop of 4 N sodiumhydroxide a bathochromic shift occurs, the spectrum showing $\lambda_{\max}$ at 212 and 286 nm, which is also in accordance with a proanthocyanidin nature (under similar conditions proanthocyanidin B2 shows $\lambda_{\max}$ 216 and 289 nm; epicatechin $\lambda_{\max}$ 207 and 298 nm). Field Desorption Mass-spectrometric analysis displayed a mass of 562 for compound C. The difference of 16 mass-units between the parent peaks of proanthocyanidin B2 (M^+ 578) and component C indicates the molecule of the latter compound to have one oxygen atom less than that of the former.

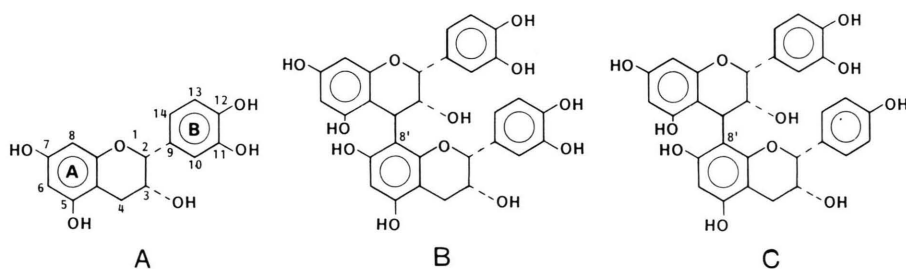
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Table I. NMR data of substance C.

δ [ppm]	Shape	Coupling	Constants [cps]	Assignment
7.7–8.3	multiplet			arom OH
7.3–6.7	multiplet			H10, 13, 14, 10', 11', 13', 14'
6.1–5.9	multiplet			H6, 8, 6'
5.1	broad singlet			H4
4.95	broad singlet			H2'
4.75	doublet		2	H2
4.3	multiplet			H3'
4.0	octet		1.1, 2.0, 5.5	H3
3.7	2× doublet		5.5, 5.5	alif OH
2.9	quartet		3.6, 15.7	H4'
2.7	quartet		4.7, 15.7	H4'

Fig. 1. Part of ^1H NMR spectrum of substance C, showing signals from B-phenyl rings.

The proton NMR data of compound C and the assignments are shown in Table I. The proton NMR spectrum of compound C shows an excessive similarity with that of proanthocyanidin B2 [7]. This finding also proves the close relationship in molecular structure between compound C and proanthocyanidin B2. The only difference is found in the coupling pattern between 6.5 and 7.5 ppm (Fig. 1, Table I). These resonance signals correspond to the aromatic protons of the B- and B'-rings in both compounds. In case of compound C integration of the multiplets between 6.5 and 7.5 ppm (Fig. 1) showed that seven protons were involved. Since cyanidin is formed from acid treatment of compound C we know that its "upper" monomere has an O-dihydroxy substitution (at position C₄ and C₃) in the B-ring. Hence the "lower" flavanolic part of compound C bears the monohydroxylated B'-ring. The double doublet, showing signals at 7.28 and 6.75 ppm (Fig. 1) with coupling constants of 8.5 cps, is consistent with a para substitution of the hydroxy group at the B'-ring. On the basis of these data we propose compound C to have the molecular structure shown below, which can be indicated to be 11'-deoxyproanthocyanidin B.



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