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# A BIOLOGICALLY-ACTIVE PROCYANIDIN FROM *MACHAERIUM FLORIBUNDUM**

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**Key Word Index**—*Machaerium floribundum*; Leguminosae; proanthocyanidin; procyanidin; antibacterial activity.

**Abstract**—A procyanidin was isolated from *Machaerium floribundum* and characterized by its $^{13}C$ NMR spectrum. The procyanidin consists of an average of four units, $M_n$ = 1150, and the stereochemistry of the heterocyclic ring system is *cis*. The procyanidin inhibited the growth of *Pseudomonas maltophilia*.

## INTRODUCTION

Condensed cotton tannin has been found to be an antibiotic chemical for the tobacco budworm *Heliothis virescens* Fabricius [1–3]. When fed to tobacco budworm hatchling larvae in diets, the $ED_{50}$ value (per cent of diet) of condensed cotton tannin was 0.15 [2]. Other condensed tannins that occur in human and animal food sources have deleterious nutritional effects [4–9].

In a preliminary search (unpublished data) to identify plants having biological activity for insects, extracts of *Machaerium floribundum* Bentham inhibited the growth of *Pseudomonas maltophilia* Hugh et Ryschenkow and *Enterobacter cloacae* (Jordan) Hormaeche et Edwards, two bacteria isolated from the gut of *H. virescens* and *Heliothis zea* Boddie, the corn earworm [10].

## RESULTS AND DISCUSSION

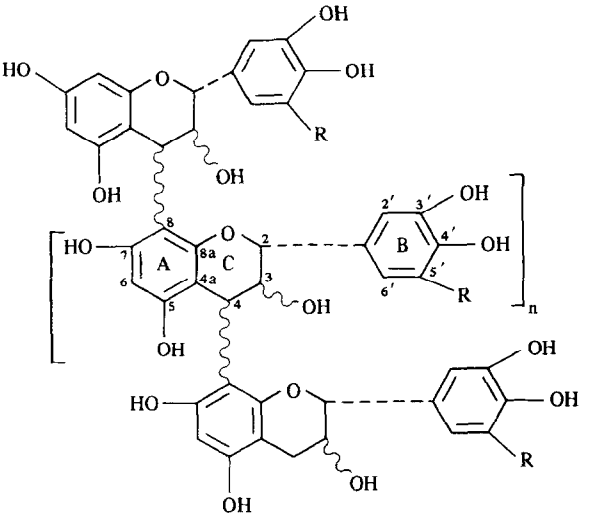
A procyanidin was isolated from an alcoholic extract of *M. floribundum* by fractionation on Sephadex LH-20 with methanol–water (7:3). Acid hydrolysis and subsequent TLC gave evidence for cyanidin but not for delphinidin. Structural elucidation was achieved by evaluating the $^{13}C$ NMR spectrum with the criteria of Czochanska *et al.* [11]: (i) the ratio of procyanidin [PC (**1a**)] to prodelphinidin [PD (**1b**)] units; (ii) the stereochemistry of the heterocyclic ring of the monomer units; and (iii) the number average molecular weight (MW).

The general structure for the isolated polymer (**1**), consistent with the $^{13}C$ NMR spectrum (Fig. 1) was that of a polymer consisting of 4 → 8 linked flavan-3-ol monomer units [11–13]. The observed resonances are broadened because of the high molecular weight and the variety of unresolved chemical shifts. Assigned chemical shifts for resonances of **1** and related compounds (Table 1) occur in two regions. Region A, 30–90 ppm, includes shifts for heterocyclic ring carbons, and region B, 90–160 ppm, includes shifts for aromatic carbons.

The ratio of the peak areas of the 146 ppm (C-3′ and C-4′ of the PC B-ring) in region B has previously been employed [11] to determine the ratio of procyanidin to prodelphinidin (PC:PD) monomer units. There were no detectable signals at 146 ppm (C-5′, PD) or 108 ppm (C-2′, PD). Signals were observed at 143 ppm (C-5′, PC) and 115 ppm (C-2′, PC), however, indicating that **1** has a hydrogen at C-5′ and therefore contains cyanidin monomer units only.

It has also been shown [11] that PC and PD units possess either of the heterocyclic ring stereochemistries 2*R*,3*R*,4*R* (*cis*) or 2*R*,3*S*,4*S* (*trans*). The C-2 in a *cis*-unit has a chemical shift at ~ 77 ppm while that in a *trans*-unit occurs at approximately 84 ppm (Table 1). Because of the absence of a detectable signal at 84 ppm and the presence of a signal at ~ 76 ppm, it can be deducted that **1** is primarily, if not completely, composed of 2,3-*cis*-stereochemistry.

The C-3 chemical shifts of the flavan-3-ol terminal



**1a** R=H, procyanidin units (PC)
**1b** R=OH, prodelphinidin units (PC)

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Table 1. $^{13}C$ NMR chemical shifts of proanthocyanidin polymers*

| Polymer | C-2 | C-3 | C-4 | C-4a | C-5 | C-6 | C-7 | C-8 | C-8a | C-1′ | C-2′ | C-3′ | C-4′ | C-5′ | C-6′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ribes sanguineum* condensed tannin† | 84 | 73 | 38 | ~107 | ~157 | 98 | ~157 | 107 | ~157 | 133 | 108 | 146 | 134 | 146 | 108 |
| *Cydonia oblonga* condensed tannin‡ | 77 | 72 | 37 | 102 | ~156 | 98 | ~156 | 107 | ~156 | 132 | 115 | 145 | 145 | 116 | 119 |
| *Machaerium floribundum* condensed tannin (**1**) | ~76 | 71 | 36 | ~107 | ~154 | 96 | ~154 | ~107 | ~154 | 131 | 115 | 143 | 143 | 115 | 120 |

*$\delta(^{13}C)$ relative to TMS.
†A PD polymer with 2,3-*trans* stereochemistry [11].
‡A PC polymer with 2,3-*cis* stereochemistry [11].

groups occur at 65–66 ppm, while the C-3 chemical shifts for monomer units are found at 72–73 ppm. On the basis of the chemical shift of C-3 for the *trans*-unit (65–66 ppm), it is also *cis*, i.e. epicatechin. The number of terminal units compared to that of interior units can be determined by finding the ratio of the monomer to terminal C-3 signals by integration. Based on a ratio of three monomer units to one terminal unit, the number average molecular weight for **1** is 1150 ($n = 2$).

The antibacterial activity of **1** against *P. maltophilia* and *E. cloacae* was compared with that of a condensed tannin obtained from cotton, *Gossypium hirsutum* L. (strain DES-06), cyanidin chloride, (+)-catechin, and (−)-epicatechin. The bioassay results (Table 2) show that

Table 2. Antibacterial test results for plant polyphenols

| Compound | Zone width (mm) | |
|---|---|---|
| | *P. maltophilia* | *E. cloacae* |
| *M. floribundum* condensed tannin | 13.0 | NA* |
| Condensed cotton tannin | 10.3 | 10.3 |
| Cyanidin chloride | NA | NA |
| (+)-Catechin | 8.8 | 9.3 |
| (−)-Epicatechin | 7.0 | 7.0 |

*NA = no activity.

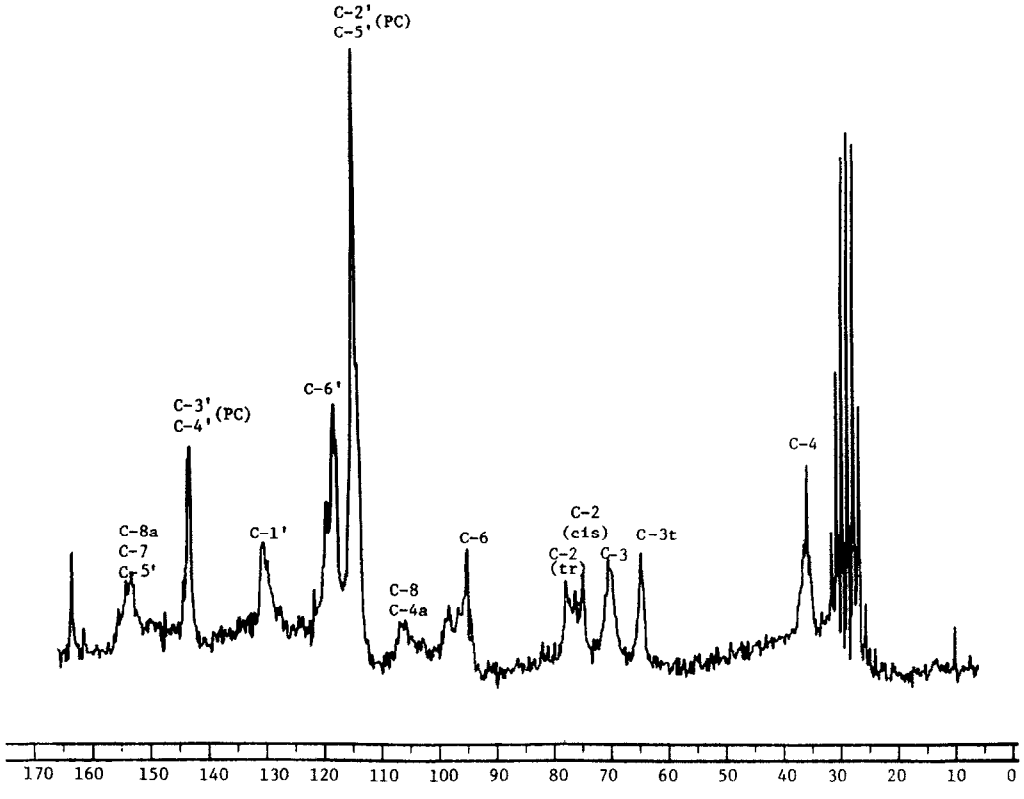


Fig. 1. $^{13}C$ NMR spectrum of *Machaerium floribundum* procyanidin

the procyanidin from *M. floribundum* inhibited the growth of *P. maltophilia*, but not that of *E. cloacae*, while the cotton proanthocyanidin inhibited the growth of both bacteria. The cotton proanthocyanidin (unpublished data) was a group of polymers with molecular weights ranging from 1500 to 6000, a prodelphinidin : procyanidin ratio of from 1.8 to 3.7, and the stereochemistry of the heterocyclic ring system was primarily *cis*. The differences in the activities of the two proanthocyanidins may be attributed to their structural differences. Cyanidin did not inhibit the growth of either of the two bacteria, while the catechins showed activity against both bacteria.

## EXPERIMENTAL*

*Extraction of plant material. M. floribundum* (woody stems and bark; 200 g), collected by the Institute for Botanical Exploration of Mississippi State University, was freeze-dried and ground to give a coarse brown powder. The powder was extracted for 18 hr with $CH_2Cl_2$ to give fraction 1, and the subsequent residue was extracted for 18 hr with absolute EtOH to give fraction 2. The EtOH extract was partitioned between $CHCl_3$ and $H_2O$ (1:1) to give $CHCl_3$ (fraction 3) and $H_2O$ (fraction 4) phases. The $CHCl_3$ phase was then partitioned between hexane and MeOH–$H_2O$ (9:1) to give hexane (fraction 5) and MeOH–$H_2O$ (fraction 6) phases. The $H_2O$ (fraction 4) phase was partitioned between $CHCl_3$ and EtOH (1:1) to give $CHCl_3$–EtOH (fraction 7) and $H_2O$ (fraction 8) phases. Fraction 8 showed antibacterial activity for both *P. maltophilia* and *E. cloacae*.

*Chromatography of fraction* 8. Fraction 8 was chromatographed on Sephadex LH-20 in MeOH–$H_2O$ (7:3) to give six subfractions. On adding 0.2 ml of *n*-BuOH–HCl (19:1) and heating at 99° for 1.5 hr, the soln turned dark red, an indication of a polymeric proanthocyanidin. The other fractions also contained proanthocyanidin polymer (6.71 g; 3.36%).

*TLC.* The hydrolysate of subfraction 6 was chromatographed on polyamide layers in MeOH–$HCONMe_2$ (19:1). The chromatogram was developed by spraying with natural product A reagent (5% β-aminoethyl diphenylborinate in EtOH). Cyanidin was identified by co-chromatography with an authentic standard.

*$^{13}$C NMR spectroscopy of* **1**. The $^{13}$C NMR spectrum was obtained with a Varian CT-20 spectrometer at 20 MHz and 37° using an 8K data table. The spectrum was obtained for a near-saturated soln (> 100 mg in 1.5 ml) of the polymer in $D_2O$–$d_6$-$Me_2CO$ (1:1), using 45° pulses at 0.2 sec intervals during continuous proton decoupling. Spectrometer conditions and the assumption of the same $T_1$ and $\eta$ for similar aliphatic and aromatic carbons for comparing peak areas have been shown to be valid for proanthocyanidin polymers [11–13].

*Bioassay.* Samples were screened for antibacterial activity against the pathogens *P. maltophilia* and *E. cloacae*, with bactosensitivity discs (BBL). Each fraction to be tested was dissolved in an appropriate solvent (1 mg/20 μl). A 20 μl aliquot of the soln was applied to a blank sensitivity disc, and the solvent was allowed to evaporate overnight. Three replications were employed for each sample. A suspension of each bacterium in a 0.85% saline soln was used to streak Petri plates (15 mm × 100 mm diameter) containing solidified tryptic-soy agar. The sensitivity discs were placed on the plates, no closer than 10–15 mm from each other (no more than 7 discs per plate), and the plates were incubated overnight at 37°. Inhibition was determined by measuring the diameter of the clear zone (if present) around the disc.

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