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# ISOMERIC LEUCOFISETINIDINS FROM *ACACIA MEARNSII*

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**Abstract**—Closer examination of the heartwood extract of the black wattle (*Acacia mearnsii*) has shown that the flavandiol, (+)-mollisacacidin (2,3-*trans*-3,4-*trans* configuration), is accompanied in low concentration by the *trans–cis* and *cis–cis* isomers. These isomers have been isolated and fully characterized.

## INTRODUCTION

(+)-Mollisacacidin [(+)-7,3′,4′-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol, leucofisetinidin] is the predominant monomeric heartwood component of the commercially-afforested black wattle, *Acacia mearnsii.*[1] It has also been identified in numerous *Acacia* species indigenous to South Africa and was shown to be identical by Clark-Lewis and Mitsuno to the flavandiol from *Gleditsia japonica*[2]. The enantiomer, (−)-leucofisetinidin, occurs in *Schinopsis quebracho colorado* wood[3,4] and these two compounds represented the first example of the occurrence of an enantiomorphous pair of flavonoids in nature.

Originally, mollisacacidin was assigned the 2,3-*trans*-3,4-*cis* configuration on the grounds that it formed an acidic borate complex[1] and because it afforded the isopropylidene derivative in high yield.[5] Subsequent investigations[6,7] have shown that the compound has the 2,3-*trans*-3,4-*trans* relative configuration (2R:3S:4R absolute configuration).

The flavan-3,4-diols possess three asymmetric centres therefore eight stereoisomeric forms are theoretically possible. These stereoisomeric forms frequently occur in the same plant source, as for example in the case of melacacidin from *A. melanoxylon*[8] (two forms) and teracacidin from *A. auriculiformis*[9] (three forms). The heartwood of *Guibourtia coleosperma*[10] contains the (−)-*cis–cis* (2R:3R:4R), (−)-*cis–trans* (2R:3R:4S) and probably the (+)-*trans–cis* (2R:3S:4S) isomers of leucofisetinidin. The latter isomer has subsequently been obtained in good yield from the wood of *Colophospermum mopane.*[11] Five of the eight possible

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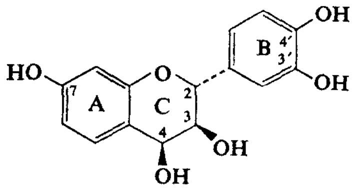
stereoisomers of leucofisetinidin are thus known to exist in nature. Epimerization under pressure of (+)-mollisacacidin and (−)-leucofisetinidin has led to the isolation of seven synthetic diastereoisomeric forms—the (−)-*cis–trans* form alone not being obtained.[10, 12]

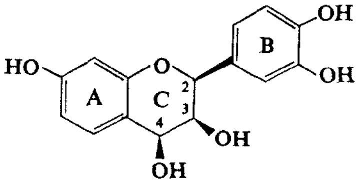
## RESULTS AND DISCUSSIONS

Re-examination of the enriched heartwood extract of black wattle has now revealed the presence of the (+)-2,3-*trans*-3,4-*cis* (I) and (+)-2,3-*cis*-3,4-*cis* (II) isomers along with (+)-mollisacacidin. The *cis–cis* isomer was separated from mollisacacidin by repeated preparative paper chromatography. The same technique could not be employed for the *trans–cis* isomer since it coincides with mollisacacidin on two-dimensional paper chromatograms. Separation was, however, effected by fractional crystallization.



(I)

2R : 3S : 4S



(II)

2S:3S:4S

Comparison with stereoisomers of known absolute configuration established the stereochemistry of the two new isomers. The *trans–cis* isomer thus has the 2R:3S:4S absolute configuration and the *cis–cis* isomer the 2S:3S:4S configuration. These stereochemical assignments are based on an NMR comparison of the size of the coupling constants between the three heterocyclic protons on ring C. Chemical shifts and coupling constants are shown in Table 1.

Rajadurai,[13] Leela and Sastry,[14] and more recently Nayudamma[15] and his co-workers, have claimed the isolation of new isomers of leucofisetinidin from *Pithecolobium dulce*, *Caesalpania coriaria* and *Cassia marginata* respectively. The properties of these leucofisetinidins are not in agreement with any of the eight known isomers of leucofisetinidin. NMR data are not given for these compounds and it is probable that they represent mixtures of different isomers.

The condensed tannins present in wattle heartwood are all based on the leucofisetinidin nucleus and the natural association of (+)-mollisacacidin with these tannins is the basis for the theory that mollisacacidin is the natural tannin precursor. Evidence in support of this comes from the fact that one of the three crystalline bileucofisetinidins[16] isolated from wattle wood is in fact composed of two 4,6-linked all-*trans* leucofisetinidin moieties. The remaining two bileucofisetinidins have the two $C_{15}$ units linked in a *trans–cis*:*trans–trans* and all-*trans–cis* fashion[16] so that the present isolation of *trans–cis* leucofisetinidin in wattle wood is not unexpected.

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TABLE 1. NMR DATA FOR DIASTEREOISOMERIC 3,4-DIACETOXY-7,3′4′-TRIMETHOXYFLAVANS

| | Chemical shifts, $\tau$ values | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Me (acetyl) | | Me (methoxyl) | | H | | | |
| Compound | 3 | 4 | 7 | 3′+4′ | 2 | 3 | 4 | 5 |
| (−)*cis–cis* isomer from *Guibourtia coleosperma* | 8·06 | 7·89 | 6·20 | 6·10 | 4·70 | 4·36 | 3·70 | ~2·82 |
| (+)*cis–cis* isomer from epimerization | 8·05 | 7·88 | 6·19 | 6·09 | 4·69 | 4·33 | 3·67 | ~2·83 |
| (+)-*cis–cis* isomer from *Acacia mearnsii** | 8·08 | 7·90 | 6·20 | 6·10 | 4·73 | 4·38 | 3·72 | 2·91 |
| (+)-*trans–cis* isomer from *Colophospermum mopane* | 8·14 | 7·84 | 6·21 | 6·08 | 4·76 | 4·45 | 3·78 | 2·74 |
| (+)-*trans–cis* isomer from *Acacia mearnsii** | 8·16 | 7·86 | 6·23 | 6·10 | 4·79 | 4·52 | 3·83 | 2·79 |

| | Spin–spin coupling constants for 2, 3 and 4 protons (C/S) | |
|---|---|---|
| | $J_{2,3}$ | $J_{3,4}$ |
| (−)-*cis–cis* isomer from *Guibourtia coleosperma* | < 1 | ~4·0 |
| (+)-*cis–cis* isomer from epimerization | 0·9 | 4·4 |
| (+)-*cis–cis* isomer from *Acacia mearnsii* | 1·0 | 4·3 |
| (+)-*trans–cis* isomer from *Colophospermum mopane* | 10·4 | 3·4 |
| (+)-*trans–cis* isomer from *Acacia mearnsii* | 10·4 | 3·6 |

* Recorded on a high resolution instrument at 100 Mc, others at 60 Mc.

## EXPERIMENTAL

Methanol extraction of wattle heartwood drillings (7·7 kg) yielded 300 g solid material. This was partitioned between EtOAc and $H_2O$ (1:1) in five 2-l. separating funnels. From six such runs 110 g enriched material was obtained by combining the contents of funnels 3, 4 and 5. The enriched extract was partitioned in an automatic, 50 ml underphase, Craig countercurrent machine using $H_2O$:*sec* BuOH:isohexane (5:3:2). Tubes 58–76 (16 g) contained the bulk of the leucofisetinidin fraction. Chromatographic separation (2% HOAc) on eighty-five sheets of Whatman No. 3 paper showed the presence of a band of high concentration at $R_f$ 0·60 and one of much lower concentration at $R_f$ 0·43. These two bands were cut out and eluted with 70% EtOH. From the higher $R_f$ band 7·9 g of buff solid were obtained while the lower $R_f$ band yielded 1·3 g of brown amorphous material.

### *Higher $R_f$ Fraction*

This fraction was re-run on fifty-two sheets of paper (2% HOAc) and from the eluates 2·5 g of white needles, m.p. 126–130°, and identical in all other respects with (+)-mollisacacidin, were obtained. The mother liquor was concentrated to a small volume (2 ml) and kept at 0° for 5 days when a further 100 mg of (+)-mollisacacidin separated. Concentration of the mother liquor of these crystals gave 1·5 g of residue which was purified by chromatography on fifteen Whatman No. 3 sheets (2% HOAc). The eluates were concentrated (680 mg) and methylated with diazomethane. From acetone white needles (180 mg) of m.p. 180–182° were obtained. Typically, these crystals are virtually insoluble in ethanol. Recrystallization from acetone raised the m.p. to 186°, $[\alpha]_D^{21} = +46·5°$ (C=0·2 in acetone) and a mixed m.p. with authentic (+)-7, 3′,4′-trimethoxy-2,3-*trans*-flavan-3,4-*cis* diol,[11] $[\alpha]_D^{21} = +45·7°$ (C=0·4 in acetone), showed no depression. (Found: C, 64·9; H, 6·2; OMe, 28·5. Calc. for $C_{18}H_{20}O_6$: C, 65·1; H, 6·1; OMe, 28·0 per cent.) Examination by electrophoresis showed that it had a positive migration in agreement with its stereochemistry.[11]

Acetylation of the trimethyl ether gave an amorphous white solid, m.p. 80°. The same behaviour is shown by the corresponding authentic *trans–cis* derivative. (Found: C, 63·4; H, 5·9. Calc. for $C_{22}H_{24}O_8$: C, 63·5; H, 5·8 per cent.) The NMR spectrum of this trimethylether diacetate derivative is shown in Table 1.

### *Lower $R_f$ Fraction*

This fraction was re-chromatographed on twenty-five sheets of Whatman No. 3 paper (2% HOAc). Solid material from the eluates (350 mg) still contained bi- and trileucofisetinidins as contaminants but these were effectively removed by partition separation of eight prewashed ($H_2O$) Whatman No. 3 sheets using $H_2O$ saturated *sec* BuOH as solvent. From the eluates fine white needles (27 mg), m.p., 136–138°, $[\alpha]_D^{20} = +49{\cdot}0°$ ($C = 0{\cdot}4$ in acetone–$H_2O$) were obtained. Authentic (+)-7,3′,4′-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol[12] had m.p. 146–150°, $[\alpha]_D^{20} = +48{\cdot}5°$ ($C = 0{\cdot}8$ in acetone–$H_2O$) and the two compounds were superimposable on two-dimensional paper chromatograms.

The free diol (19·5 mg) was methylated with diazomethane to give white needles, 11 mg, m.p. 127–129°. Mixed m.p. with authentic material, m p. 134°, gave m p. 132–134°. Acetylation yielded the crystalline diacetate ($M^+$, 416, by mass spectrometry) m.p. 152°, undepressed on admixture with authentic material. (Found: C, 63·2; H, 5·9. Calc. for $C_{22}H_{24}O_8$: C, 63 5; H, 5 8 per cent.) Details of the NMR spectrum of the trimethylether diacetate are shown in Table 1.

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