Flavonol glycosides from the leaves of *Acacia mangium* and related species

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Abstract. Several flavonol glycosides have been isolated from the leaves of *Acacia mangium*, *A. auriculiformis*, *A. richii* and *A. mangium* × *A. auriculiformis*. *A. mangium* was characterised by the presence of the 3-glucoside of quercetin, quercetin-3-diglucoside in addition to kaempferol-3,7-dirhamnoside and kaempferol-7,4'-digalactoside. On the other hand, *A. richii* contains myricetin-3-glucoside and kaempferol-3-dixyloside whereas *A. auriculiformis* contains isorhamnetin and quercetin-7-glucoside. These compounds have hitherto never been reported from *Acacia*.

Abstrak. Beberapa flavonoid glikosida telah diasingkan daripada daun *Acacia mangium*, *A. auriculiformis*, *A. richii* dan *A. mangium* × *A. auriculiformis*. *A. mangium* telah dicirikan dengan kehadiran kuersetin-3-glukosida, kuersetin-3-diglukosida dan juga kaemferol-3,7-dirhamnosida dan kaemferol-7,4'-digalaktosida. *A. richii* sebaliknya mengandungi mirisetin-3-glukosida dan kaemferol-3-dizilosida, manakala *A. auriculiformis* pula mengandungi isorhammetin dan kuersetin-7-glukosida. Sebatian-sebatian ini belum pernah lagi di laporkan daripada *Acacia*.

Key word: *Acacia*; Leguminosae; leaves; flavonoid glycosides

Introduction

Some *Acacia* species have been investigated for their flavonoids [4, 9] and these have been correlated with systematics and geography. Some triterpenoids, saponin [6, 10], coumarins, tannins, carbohydrates, alkaloids and/or nitrogenous bases [12] and cyanogenic compounds have also been reported [8]. In addition these substances have significant biological activities [12] and might play an important role in the adaptation of plants growing in tropic habitats. The aim of the present work is to analyze and identify the flavonoid glycosides from the leaves of *Acacia*.

Materials and Method

Plant material

Leaves were analysed from freshly dried plant material collected from Forest Research Institute (FRIM), Kepong.

Methods of flavonoid analysis

The flavonoids in the leaves of *Acacia* samples were surveyed by means of 2-dimensional paper chromatography and following standard procedures [3, 5, 7]. In addition to spectral techniques, the flavonoids were identified by PC cochromatography of the glycosides and products of enzyme hydrolyses in BAW (*n*-butanol-acetic acid-water; 4:1:5) and 15%HOAc (15% acetic acid). The aglycones were identified by TLC cochromatography in BAW and HOAc while the sugars were identified by PC cochromatography in BEW (*n*-butanol-ethanol-water; 4:1:2.2), BAW, PhOH (phenol-water) and TBPW (toluene- *n*-butanol-pyridine-water; 5:1:3:3). The UV spectral values were in agreement with published results [5]. Two dimensional paper chromatograms of leaf extracts of hybrid, *A. auriculiformis* × *A. mangium* was compared with those from *A. mangium* and *A. auriculiformis*. Additionally, spots were eluted from 2D-chromatograms on thick paper of hybrid extracts and were then compared with components isolated from the parents.

Results and Discussion

A total of 17 flavonoid constituents were detected on two dimensional chromatograms. The solvents were *n*-butanol, glacial acetic acid and water (BAW 4:1:5) and 15% glacial acetic acid (HOAc). In order to identify some of the components found on the 2-dimensional chromatograms, the compounds of four samples (fresh plants) were studied in more detail. From these samples 13 compounds were obtained in a more or less pure state by means of preparative layer chromatography. The common aglycones were identified by means of *R*ₚ values and colour reaction in UV light when compared with standard markers. In acid-hydrolysed extracts, the flavones were
recognized by their distinct dark yellow spots on paper chromatograms in UV light. When fumed with ammonia vapour they became bright yellow. The flavonols appeared yellow in UV light before and after fuming with ammonia. For complete identification of flavonoid glycosides, samples were separated in one-dimensional chromatograms of direct extracts and then pure flavonoid identified using standard methods [3, 5, 7]. These included: complete and mild acid hydrolysis, hydrogen peroxide oxidation, enzymic hydrolysis, cochromatography and UV spectrophotometry. Standard solvents used for chromatography were BAW, PhOH, HOAc and water [7].

Table 1 shows the $R_f$ values, colour reactions and the UV absorption spectra of flavonoids isolated from the leaves of Acacia samples. It was found that the parental components appeared additively in the hybrid (Fig. 1).

Figure 1: 2D-PC of flavoid spots from the leaf extracts of Acacia species
Table 1: Spectral and Rf properties of flavonoids of *Acacia mangium* and related species

<table>
<thead>
<tr>
<th>Flavonoids detected in leaf</th>
<th>Source</th>
<th>Rf in BAW</th>
<th>H2O</th>
<th>15%HOAc</th>
<th>PhOH</th>
<th>80%MeOH</th>
<th>NaOAc</th>
<th>H3BO3</th>
<th>NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myricetin</td>
<td>Ar</td>
<td>74</td>
<td>30</td>
<td>46</td>
<td>38</td>
<td>352, 258</td>
<td>263</td>
<td>374</td>
<td>377</td>
</tr>
<tr>
<td>3,7-diglucoside (2)</td>
<td>Ar, Am, Hy</td>
<td>74</td>
<td>30</td>
<td>47</td>
<td>43</td>
<td>353, 264</td>
<td>269</td>
<td>375</td>
<td>387</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Ar</td>
<td>67</td>
<td>40</td>
<td>13</td>
<td>58</td>
<td>347, 270</td>
<td>268</td>
<td>363</td>
<td>365</td>
</tr>
<tr>
<td>7-glucoside (3)</td>
<td>Ar, Am, Hy</td>
<td>60</td>
<td>40</td>
<td>13</td>
<td>58</td>
<td>347, 270</td>
<td>268</td>
<td>363</td>
<td>365</td>
</tr>
<tr>
<td>3,7-dirhamnoside (5)</td>
<td>Am</td>
<td>78</td>
<td>56</td>
<td>48</td>
<td>59</td>
<td>347, 270</td>
<td>268</td>
<td>363</td>
<td>365</td>
</tr>
<tr>
<td>7,4'-digalactoside (6)</td>
<td>Am</td>
<td>79</td>
<td>49</td>
<td>48</td>
<td>60</td>
<td>346, 257</td>
<td>270</td>
<td>363</td>
<td>367</td>
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<tr>
<td>4'-galactoside (8)</td>
<td>Hy</td>
<td>63</td>
<td>66</td>
<td>74</td>
<td>54</td>
<td>375, 265</td>
<td>272</td>
<td>359</td>
<td>373</td>
</tr>
<tr>
<td>3-glucoside (9)</td>
<td>Am, Hy</td>
<td>83</td>
<td>31</td>
<td>51</td>
<td>66</td>
<td>350, 263</td>
<td>271</td>
<td>360</td>
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<tr>
<td>3,7-diglucoside (10)</td>
<td>Hy</td>
<td>72</td>
<td>55</td>
<td>65</td>
<td>64</td>
<td>357, 265</td>
<td>271</td>
<td>353</td>
<td>352</td>
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<td>Quercetin</td>
<td>Am</td>
<td>61</td>
<td>68</td>
<td>56</td>
<td>53</td>
<td>355, 287</td>
<td>273</td>
<td>374</td>
<td>377</td>
</tr>
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<td>3-diglucoside (4)</td>
<td>Am</td>
<td>72</td>
<td>28</td>
<td>39</td>
<td>59</td>
<td>354, 258</td>
<td>270</td>
<td>372</td>
<td>375</td>
</tr>
<tr>
<td>3-glucoside (11)</td>
<td>Am</td>
<td>74</td>
<td>72</td>
<td>45</td>
<td>57</td>
<td>345, 268</td>
<td>275</td>
<td>344</td>
<td>373</td>
</tr>
<tr>
<td>3'-methyl ether (12)</td>
<td>Am</td>
<td>65</td>
<td>05</td>
<td>10</td>
<td>54</td>
<td>353,280</td>
<td>277</td>
<td>370</td>
<td>375</td>
</tr>
</tbody>
</table>

Am = *Acacia mangium*; Au = *A. auriculiformis*; Ar = *A. richii*; Hy = hybrid *A. mangium* x *A. auriculiformis*.

BAW = n-BuOH-HOAc-H2O (4:1:5), H2O = water; 15%HOAc = 15% acetic acid; H3BO3 = boric acid; NaOAc = natrium acetate; NaOH = natrium hydroxide

In the present study kaempferol, myricetin and quercetin were the only flavonols detected in *Acacia* samples investigated. This is not unexpected as flavonols were reported to be common constituents of the genus *Acacia* than the related flavones luteolin and apigenin [4]. Myricetin-3,7-diglucoside, kaempferol-7-glucoside and kaempferol-3-glucoside were detected in all samples except in *A. richii* which contained myricetin-3-glucoside (figure 2) and kaempferol-3-dixyloside. Kaempferol-3-glucoside was isolated before from the leaves of *A. arabica* [2].

Quercetin was identified in three samples investigated as its 3-diglucoside, 3-glucoside, 3-galactoside, 7-glucoside and 3'-methyl ether (figure 3, isorhamnetin) (after hydrolysis) (Table 1). Quercetin 3-glucoside and quercetin 3-diglucoside was detected in *A. mangium* whereas isorhamnetin and quercetin-7-glucoside was detected in *A. auriculiformis*. The present discovery of quercetin-3-glucoside, quercetin-3-galactoside, quercetin-7-glucoside, isorhamnetin and myricetin-3-glucoside in the samples investigated are also not unexpected since El-Mousallamy and coworkers [1] have already reported the presence of quercetin-3-glucoside and quercetin-3-galactoside in the leaves of *A. raddiana*. Also, the 3-glucoside, 7-glucoside, 3-galactoside of quercetin, myricetin-3-glucoside and isorhamnetin were earlier found in the flower of *A. latifolia* by Voirin and coworkers [11]. Furthermore, quercetin-7-glucoside was a major component whereas isorhamnetin was a minor component in *A. latifolia*.

![Figure 2: Myricetin-3-glucoside](image1.png)

![Figure 3: Quercetin-3'-methyl ether](image2.png)
From the point of view of clarifying the chemical background of hybridisation in *Acacia (A. mangium x A. auriculiformis)*, the identification of these various flavonol derivatives confirms the fact that distinctive biochemical processes in flavonoid synthesis are inherited additively from the individual parents. It is clear that *A. mangium* and *A. auriculiformis* have the ability to synthesize flavonol and probably also to add glucose to the 3- and 7- positions. *A. mangium* is able to synthesize a 7,4’-digalactoside and 3,7-dirhamnoside of kaempferol. By contrast, *A. auriculiformis* generally lacks these special enzymes, but has the added ability to methylate quercetin in the 3’-position.

**Acknowledgement**

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**References**