# THE FLAVONOIDS OF *CYANOSTEGIA ANGUSTIFOLIA* AND *CYANOSTEGIA MICROPHYLLA**

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*Cyanostegia* (Verbenaceae) is represented by four species of small shrubs all of which occur in Western Australia. Apart from *C. cyanocalyx* (F. Muell) Gardn. which is found in the Kimberley district, the species are confined to the outer wheat belt where their dense blue flowers form a striking feature of the spring landscape. The leaves of these species possess viscid coatings which attracted our attention, and incidental to a study of the associated diterpenoids we have isolated a number of flavones which are the subject of this communication.

Table 1

CHEMICAL SHIFTS OF PROTONS IN FLAVONE ACETATES

$\tau$ values in $CDCl_3$; s, singlet; q, quartet ($J_m$ 2 c/s; $J_o$ 9 c/s); d, doublet ($J_o$ 9 c/s); m, multiplet; coupling constants are derived from first-order analysis

| Compound | A Ring | | B Ring | | | | Methoxyl | Acetoxyl |
|---|---|---|---|---|---|---|---|---|
| | H6 | H8 | H2′ | H6′ | H3′ | H5′ | | |
| (Ib) | s 3·15 | | s 2·11 | q 2·18 | | d 2·75 | 6·11, 6·04, 5·96 | 7·65, 7·62, 7·54 (5-OAc) |
| (IIb) | s 3·20 | | 1·83* | | 2·73* | | 6·01, 6·17 | 7·63, 7·66, 7·55 (5-OAc) |
| (IIIb) | | s 3·15 | 1·94* | | 2·77* | | 6·17, 6·13, 6·03 | 7·66 (4′-OAc), 7·49 (5-OAc) |
| (IVb) | | s 3·16 | s 2·07 | q 2·00 | | m 2·68 | 6·07, 6·17 (2) | 7·68, 7·70, 7·51 (5-OAc) |

* $A_2B_2$ system $J_o \simeq 9 \cdot 0$ c/s.

The alkali-soluble fractions of the ether extract of *C. angustifolia* Turcz. has yielded the new pigments 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone (Ia) and 5,7,4′-trihydroxy-3,8-dimethoxyflavone (IIa), as well as 5,4′-dihydroxy-3,7,8-trimethoxyflavone previously obtained from *Ricinocarpus stylosus*.[1]

The flavone (Ia) was characterized as the triacetate (Ib) which contained resonances in the n.m.r. spectrum (Table 1) due to the protons of three acetate and three methoxyl groups. Three of the four aromatic protons gave rise to an ABC pattern indicating 3′,4′ substitution. Consistently, partial methylation of (Ia) gave the pentamethoxyflavone (Ic) identical with gossypetin pentamethyl ether.[1] The other hydroxyl groups in (Ia) were located at 4′ and 7 by alkaline degradation of the triethyl ether (Id) which gave 4-ethoxy-3-methoxybenzoic acid and 4,6-diethoxy-2-hydroxy-$\omega$,3-dimethoxyacetophenone.[2]

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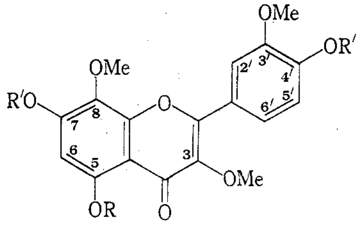
[1] Henrick, C. A., and Jefferies, P. R., *Aust. J. Chem.*, 1964, **17**, 934.

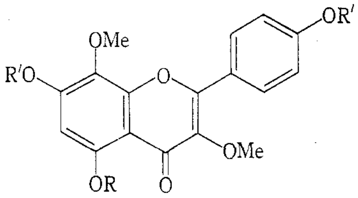
[2] Dawson, R. M., Henrick, C. A., Jefferies, P. R., and Middleton, E. J., *Aust. J. Chem.*, 1965, **18**, 1871.

*Aust. J. Chem.*, 1967, **20**, 1049–53

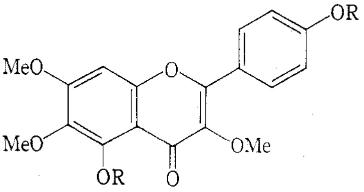
The pigment (IIa) was found to contain three hydroxyl and two methoxyl groups. The n.m.r. spectrum of the triacetate (IIb) revealed aromatic proton resonances as an $A_2B_2$ pattern and the chemical shifts of these protons are expected for a *p*-acetoxyphenyl B ring.[1] This was confirmed by alkaline degradation of the triethyl ether (IId) which gave *p*-ethoxybenzoic acid as well as 4,6-diethoxy-2-hydroxy-ω,3-dimethoxyacetophenone. The formation of flindulatin[3] (IIc) after treatment of the parent flavone (IIa) with diazomethane confirmed the oxygenation pattern.



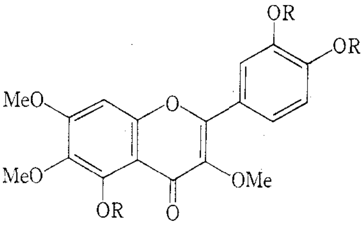
| | R | R′ |
|---|---|---|
| (Ia) | H | H |
| (Ib) | Ac | Ac |
| (Ic) | H | Me |
| (Id) | Et | Et |



| | R | R′ |
|---|---|---|
| (IIa) | H | H |
| (IIb) | Ac | Ac |
| (IIc) | H | Me |
| (IId) | Et | Et |



(IIIa) R = H
(IIIb) R = Ac



(IVa) R = H
(IVb) R = Ac
(IVc) R = Et

The alkali-soluble fractions of *C. microphylla* S. Moore gave penduletin (IIIa), previously obtained from *Brickelia pendula*[4] and *Newcastlia viscida*,[5] as well as the unknown 5,3′,4′-trihydroxy-3,6,7-trimethoxyflavone (IVa). The latter formed a triacetate (IVb) whose n.m.r. spectrum showed resonances due to three methoxyl groups in addition to an ABC pattern arising from aromatic protons. The structure (IVa) was established by conversion into the triethyl ether (IVc), which was degraded with base to give 3,4-diethoxybenzoic acid and 6-ethoxy-2-hydroxy-ω,4,5-trimethoxyacetophenone.

[3] Brown, R. F. C., Gilham, P. T., Hughes, G. K., and Ritchie, E., *Aust. J. Chem.*, 1954, **7**, 181.

[4] Flores, S. E., and Herran, J., *Tetrahedron*, 1958, **2**, 308.

[5] Dawson, R. M., Jarvis, M. W., Jefferies, P. R., Payne, T. G., and Rosich, R. S., *Aust. J. Chem.*, 1966, **19**, 2133.

The ultraviolet spectral data for the new flavones support the proposed structures and the details are listed in Table 2. The large bathochromic shift of the long-wavelength band, without a decrease in its relative intensity, observed on making the ethanolic solutions of (Ia), (IIa), and (IVa) alkaline indicates a 4′-hydroxyl group.[6] The free 7-hydroxyl group in (Ia) and (IIa) is indicated by the bathochromic shift of the short-wavelength band in the presence of fused sodium acetate in absolute ethanol.[7]

TABLE 2

U.V. SPECTRA OF FLAVONES

($\lambda_{max}$ in m$\mu$)

| Compound | EtOH | | NaOH/EtOH[a] | | EtOH/NaOAc[b] | |
|---|---|---|---|---|---|---|
| | $\lambda_{max}$ | log $\epsilon$ | $\lambda_{max}$ | log $\epsilon$ | $\lambda_{max}$ | log $\epsilon$ |
| (Ia) | 260sh | 4·33 | 276sh | 4·50 | 284 | 4·47 |
| | 277 | 4·45 | 284 | 4·52 | 326 | 4·14 |
| | 332 | 4·29 | 339sh | 4·16 | 414 | 4·30 |
| | 365 | 4·30 | 423 | 4·60 | | |
| (IIa) | 276 | 4·49 | 283 | 4·57 | 283 | 4·59 |
| | 310sh | 4·30 | 335 | 4·28 | 305sh | 4·30 |
| | 321sh | 4·29 | 412 | 4·55 | 316sh | 4·29 |
| | 361 | 4·23 | | | 402 | 4·27 |
| (IVa) | 271 | 4·29 | 280 | 4·27 | 265 | 4·39 |
| | 360 | 4·31 | 395 | 4·29 | 410 | 4·34 |
| (IVb) | 262 | 4·25 | | | | |
| | 317 | 4·37 | | | | |

[a] One drop of 4% aq. NaOH in 2·5-ml cell. [b] EtOH saturated with dry sodium acetate.

## Experimental

General experimental methods have been listed elsewhere.[8] The n.m.r. spectra were measured with a Varian A60 spectrometer (60 Mc/s) for $CDCl_3$ solutions containing tetramethylsilane as internal reference. Ultraviolet absorption spectra were determined with a Perkin–Elmer 137UV spectrophotometer.

### (*a*) *Extraction of* Cyanostegia angustifolia

Dried leaves and terminal branches (6 kg) collected near the 247-mile peg off the Great Eastern Highway, Western Australia, in November 1961 were exhausted with ether, and this extract was fractionated by washing with 8% $NaHCO_3$ and 5% NaOH. The latter solution was acidified and extracted with ether, which was washed with 8% $NaHCO_3$ and 5% $Na_2CO_3$. The material recovered from the carbonate extract eventually crystallized, and it was washed with

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[6] Jurd, L., "The Chemistry of Flavonoid Compounds." (Ed. T. A. Geissman.) Ch. 5. (Pergamon Press: Oxford 1962.)

[7] Jurd, L., and Horowitz, R. M., *J. org. Chem.*, 1957, **22**, 1618; see also Gentili, B., and Horowitz, R. M., *Tetrahedron*, 1964, **20**, 2313.

[8] Henrick, C. A., and Jefferies, P. R., *Tetrahedron*, 1965, **21**, 3219.

light petroleum and then ether. The insoluble residue (15 g) was repeatedly extracted with hot chloroform and the soluble portion (6·9 g) crystallized from methanol to give *5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone* (Ia) (2·5 g) as yellow plates, m.p. 215–217° (Found: C, 59·8; H, 4·7; OMe, 25·2. $C_{18}H_{16}O_8$ requires C, 60·0; H, 4·5; 3×OMe, 25·8%).

The chloroform-insoluble residue and the methanolic mother liquor were combined and fractionally crystallized from methanol to give *5,7,4′-trihydroxy-3,8-dimethoxyflavone* (IIa) as yellow needles (1·38 g), m.p. 242–244° and 254–256° (Found: C, 61·85; H, 4·4; OMe, 19·0. $C_{17}H_{14}O_7$ requires C, 61·8; H, 4·3; 2×OMe, 18·8%). Fractional crystallization also provided a small quantity of the more soluble 5,4′-dihydroxy-3,7,8-trimethoxyflavone, as yellow needles, m.p. 262–264° undepressed on admixture with authentic material of m.p. 266–268°.

All the pigments gave olive green colours with ferric chloride in ethanolic solution.

### (*b*) *Flavone Acetates*

(i) 5,7,4′-Trihydroxy-3,8,3′-trimethoxyflavone (Ia) was acetylated with acetic anhydride/sodium acetate (reflux) to give the light-sensitive *triacetate* (Ib) which recrystallized from methanol as needles, m.p. 143–145° (Found: C, 58·95; H, 4·7; OMe, 18·2. $C_{24}H_{22}O_{11}$ requires C, 59·3; H, 4·6; 3×OMe, 19·1%).

(ii) 5,7,4′-Trihydroxy-3,8-dimethoxyflavone (IIa) was acetylated as in (i) to give the *triacetate* (IIb) which crystallized from ethanol as needles, m.p. 167–168° (Found: C, 60·8; H, 4·4; OMe, 13·6. $C_{23}H_{20}O_{10}$ requires C, 60·5; H, 4·4; 2×OMe, 13·6%).

(iii) 5,4′-Dihydroxy-3,7,8-trimethoxyflavone was acetylated as in (i) to give the diacetate, m.p. 182–184°, unchanged on admixture with authentic material.

### (*c*) *Partial Methylation of 5,7,4′-Trihydroxy-3,8,3′-trimethoxyflavone*

The flavone (Ia; 170 mg) in methanol (105 ml) was treated with an excess of diazomethane in ether at 0° for 2 hr. The excess of reagent was destroyed by addition of acetic acid, the solvent removed, the product taken up in chloroform, and the solution washed repeatedly with 3% aq. KOH. The alkali-insoluble product (110 mg) was crystallized from ethanol to give gossypetin pentamethyl ether (Ic), m.p. 159–161° alone or mixed with an authentic sample (lit.[1] m.p. 157–158°).

### (*d*) *Ethylation of 5,7,4′-Trihydroxy-3,8,3′-trimethoxyflavone*

The pigment (Ia; 350 mg) in acetone (30 ml) was heated under reflux with $K_2CO_3$ (2·0 g) and diethyl sulphate (5·0 ml) for 23 hr. The solvent was removed in vacuum, the residue shaken with aqueous ammonia, and the neutral product (0·24 g), isolated with chloroform, crystallized from aqueous methanol to give the *triethyl ether* (Id), m.p. 110–111° (Found: C, 64·9; H, 6·6. $C_{24}H_{28}O_8$ requires C, 64·85; H, 6·35%).

### (*e*) *Alkaline Degradation of 5,7,4′-Triethoxy-3,8,3′-trimethoxyflavone*

The triethyl ether (Id; 195 mg) in ethanol (25 ml) was heated with KOH (2 g) under reflux for 12 hr under nitrogen. The solvent was removed in vacuum and the residue dissolved in water, acidified, and extracted with ether. Recovery of the acidic fraction with 8% aq. $NaHCO_3$ solution and crystallization from water gave 4-ethoxy-3-methoxybenzoic acid (67 mg), m.p. and mixed m.p. 196–197°. Evaporation of the ether solution and crystallization of the residue from light petroleum gave 4,6-diethoxy-2-hydroxy-ω,3-dimethoxyacetophenone (27 mg), m.p. and mixed m.p. 125–126°.

### (*f*) *Partial Methylation of 5,7,4′-Trihydroxy-3,8-dimethoxyflavone*

The pigment (IIa; 174 mg) in methanol (95 ml) was treated with excess diazomethane in ether at 0° for 2 hr. The solution was worked up as in (*c*) to give flindulatin (IIc; 90 mg), m.p. 159–161° and 166–168°. On admixture with an authentic sample (m.p. 167°) the mixture had m.p. 166–168°.

### (*g*) *Ethylation of 5,7,4′-Trihydroxy-3,8-dimethoxyflavone*

The pigment (IIa; 335 mg) was ethylated as in (*d*) to give a neutral product (300 mg). Crystallization from benzene/light petroleum gave the *triethyl ether* (IId) as fine needles, m.p. 128–129° (Found: C, 66·95; H, 6·4. $C_{23}H_{26}O_7$ requires C, 66·65; H, 6·3%).

### (*h*) *Alkaline Degradation of the Triethyl Ether* (*IId*)

The triethyl ether (IId; 242 mg) was treated as in (*e*). The acid fraction gave a product (52 mg) which crystallized from water as needles, m.p. 197–198° alone or mixed with authentic 4-ethoxybenzoic acid. The phenolic fraction (118 mg) crystallized from light petroleum as needles, m.p. 125–126° undepressed on admixture with an authentic sample of 4,6-diethoxy-2-hydroxy-ω,3-dimethoxyacetophenone.

### (*i*) *Extraction of* Cyanostegia microphylla

Extraction of the dried leaves and terminal branches (8 kg) as in (*a*) and recovery of the 5% NaOH extract gave an ether-insoluble precipitate (2·5 g). Crystallization from ethanol/benzene gave yellow needles of 5,4′-dihydroxy-3,6,7-trimethoxyflavone (penduletin) (IIIa), m.p. 214–216° alone or on admixture with an authentic sample. The diacetate (IIIb), prepared with acetic anhydride and perchloric acid catalyst, crystallized from acetone/light petroleum as prisms, m.p. 158–159° (lit.[4] 157–158°).

The 5% $Na_2CO_3$ extract obtained as in (*a*) was recovered and the insoluble precipitate washed with ether. Treatment of the pigment with acetic anhydride and perchloric acid catalyst gave a product (3·0 g) which was dissolved in benzene and the solution filtered through a column of neutral alumina (activity III). The eluate was crystallized from methanol to give needles of *5,3′,4′-triacetoxy-3,6,7-trimethoxyflavone* (IVb), m.p. 159–160° (Found: C, 59·6; H, 4·9; OMe, 17·6. $C_{24}H_{22}O_{11}$ requires C, 59·3; H, 4·6; 3×OMe, 19·1%).

### (*j*) *5,3′,4′-Trihydroxy-3,6,7-trimethoxyflavone* (*IVa*)

The triacetate (IVb), ethanol (35 ml), and 50% aq. $H_2SO_4$ were heated under reflux for 2 hr. Dilution of the mixture with water afforded a yellow precipitate (750 mg) which separated from aqueous methanol as yellow needles of the *flavone* (IVa), m.p. 235–236° (Found: C, 60·2; H, 4·6; OMe, 24·4. $C_{18}H_{16}O_8$ requires C, 60·0; H, 4·5; 3×OMe, 25·8%). The pigment gave an olive-green colour with ferric chloride in ethanolic solution and a magenta colour on reduction with Mg and conc. HCl in ethanol.

### (*k*) *Ethylation of 5,3′,4′-Trihydroxy-3,6,7-trimethoxyflavone*

The pigment (IVa; 400 mg) in acetone (70 ml) was heated with $K_2CO_3$ (5 g) and diethyl sulphate (4 ml) under reflux for 16 hr. The product (350 mg) recovered as in (*d*) recrystallized from benzene/light petroleum as needles of the light-sensitive *triethyl ether* (IVc), m.p. 96–97° (Found: C, 65·0; H, 6·4. $C_{24}H_{28}O_8$ requires C, 64·85; H, 6·35%).

### (*l*) *Alkaline Degradation of 5,3′,4′-Triethoxy-3,6,7-trimethoxyflavone*

The triethyl ether (IVc; 200 mg) was degraded with base as in (*e*). Recovery of the acidic fraction and crystallization from benzene/light petroleum gave needles of 3,4-diethoxybenzoic acid (65 mg), m.p. 166–167°, undepressed on admixture with an authentic sample (lit.[9] 166–167°). Recovery of the phenolic compound (119 mg) and recrystallization from light petroleum gave 6-ethoxy-2-hydroxy-ω,4,5-trimethoxyacetophenone, m.p. 82–83°, alone or on admixture with an authentic sample.

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[9] Sasaki, T., *J. pharm. Soc. Japan*, 1964, **84**, 47.