Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 789–793
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Flavanoids from *Caesalpinia pulcherrima*☆

K.V.N.S. Srinivas, Y. Koteswara Rao, I. Mahender, Biswanath Das*, K.V.S. Rama Krishna, K. Hara Kishore, U.S.N. Murty

Indian Institute of Chemical Technology, Hyderabad-500 007, India

Received 24 February 2003; received in revised form 7 May 2003

Abstract

Two new flavanoids, 5,7-dimethoxy-3′,4′-methylenedioxyflavanone and isobonducellin along with 2′-hydroxy-2,3,4′,6′-tetramethoxychalcone, 5,7-dimethoxyflavone and bonducellin were isolated from the aerial parts of *Caesalpinia pulcherrima*. The structures of the compounds were settled mainly by interpretation of their 1D and 2D NMR spectra. Isobonducellin was found to be a homoisoflavanoid containing a *cis* (*Z*)-double bond. Antimicrobial activity of the new compounds was evaluated.

Keywords: *Caesalpinia pulcherrima*; Leguminosae; Flavanoids; Homoisoflavanoids; Antimicrobial activity

1. Introduction

Caesalpinia pulcherrima (L.) Swartz is an ornamental plant found throughout India. The stems of the plant are used as abortifacient and emmenagogue while fruits are employed to cure diarrhea and dysentery. Decoction of the plant is applied to treat various infections (Ragasa et al., 2002). Earlier works reported the isolation of diterpenoids (Che et al., 1986; McPherson et al., 1986; Patel et al, 1997; Ragasa et al., 2002), peltogynoids and flavanoids (McPherson et al., 1983; Namikoshi et al., 1987; Passador et al., 1997). Some of the constituents were found to possess antitumour (Che et al., 1986; Patel et al., 1997) and antimicrobial properties (Ragasa et al, 2002). Recently we carried out the chemical investigation on the aerial parts of the plant. We report herein the isolation, structure elucidation and antimicrobial activity of two new flavanoids, 5,7-dimethoxy-3′,4′-methylenedioxyflavanone (**1**) and isobonducellin (**2**). The known flavanoids, 2′-hydroxy-2,3,4′,6′-tetramethoxychalcone (**3**) (Venturella and Bellino, 1960), 5,7-dimethoxyflavanone (**4**) (Bick et al., 1972) and the homoisoflavanoid, bonducellin (**5**) (McPherson et al., 1983) were also isolated. The occurrence of the compounds **3** and **4** in the investigated plant was observed here for the first time.

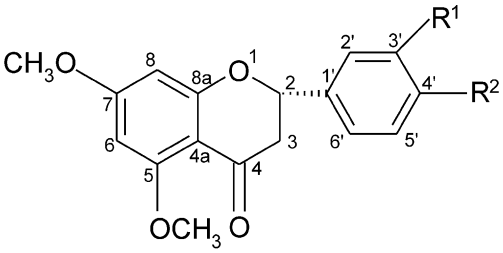
2. Results and discussion

The crude acetone extract of the aerial parts of *Ceasalpinia pulcherrima* was subjected to column chromatography over silica gel to obtain five flavanones, **1**–**5**. Compound **1** was obtained as colourless powder. It analyzed for $C_{18}H_{16}O_8$ from its mass spectrum (M^+ at m/z 328), elemental analysis and ^{13}C NMR spectrum. The spectral (IR, ^1H NMR and ^{13}C NMR) data of the compound revealed that its structure was similar to that of the known constituent, 5,7-dimethoxyflavanone (**4**) (Bick et al., 1972) but it contained an methylenedioxy group (δ 5.99, 2H, s). This group was reasonably placed at 3′,4′-position as the ^1H and ^{13}CNMR values of the protons and carbons respectively indicated that the ring A of both the compounds **1** and **4** were similar and the B ring of the former was 1′, 3′, 4′-trisubstituted (δ 6.95, 1H, d, $J=1.5$ Hz; 6.91, 1H, *dd*, $J=8.0$, 1.5 Hz; and 6.82, 1H, $J=8.0$ Hz). Thus the structure of **1** was settled as 5,7-dimethoxy-3′,4′-methylenedioxyflavanone. This structure was further confirmed from ^1H-^1H COSY, NOESY and HMBC experiments. The HMBC experiment

☆ Part 41 in the series, "Studies on Phytochemicals". Dedicated with regards to Professor (Mrs.) Asima Chatterjee on the occasion of her 86th birthday.

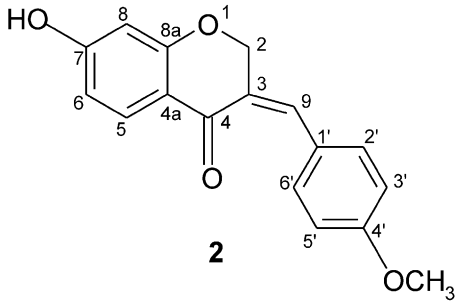
* Corresponding author. Tel.: +91-40-7193434; fax: +91-40-7160512.
E-mail address: biswanathdas@yahoo.com (B. Das).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00325-X

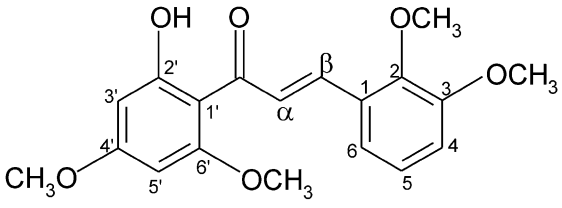


1 R^1, R^2 = $-OCH_2O-$

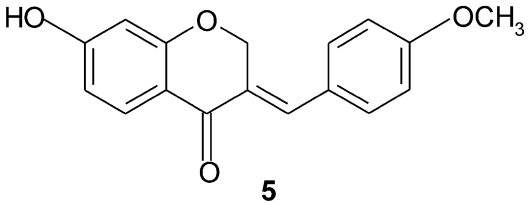
4 $R^1 = R^2 = H$



2



3



5

showed that the carbonyl group at C-4 (δ 189.2) was related to H-2 (δ 5.31, m) which was again correlated to C-2′ (δ 106.8). The C-2′ also showed correlation to H-6′ (δ 6.91, dd, $J = 8.0$, 1.5Hz) and the latter to C-4′ (δ 147.9). The protons of the methylenedioxy group (δ 5.99, 2H, s) were related to C-4′ as well as to C-3′ (δ 148.0). This C-3′ was again related to H-5′ (δ 6.82, d, $J = 8.0$ Hz). The HMBC spectrum also showed the correlation of the two methoxy groups at δ 3.89 and 3.82 (3H each, s) with C-5 (δ 162.3) and C-7 (δ 164.8) respectively. The NOESY experiment suggested that H-6 (δ 6.10, d, $J = 1.5$ Hz) was related to both the methoxy groups while H-8 (δ 6.14, d, $J = 1.5$ Hz) to only one methoxy group at C-7. The positions of the methylenedioxy group at C-3′, C-4′ and the two methoxy groups at C-5 and C-7 were thus unambigueously confirmed.

The CD measurement suggested (Iinuma et al., 1994) C-2 S-configuration of the flavanone **1**.

Compound **2** was obtained as a yellow crystalline solid. Its molecular formula was assigned as $C_{17}H_{14}O_4$ from its mass spectrum (M^+ at m/z 282), elemental analysis and ^{13}C NMR spectrum. Its IR spectrum showed characteristic absorption (ν_{max} 3127, 1602, 1572, 1509, 1475 cm^{-1}) of an unsaturated homoisoflavanone containing hydroxyl group (s) (McPherson et al., 1983). The ^1H NMR spectrum of the compound was very informative regarding its structure. In the aromatic region three protons appearing at δ 7.84, 1H, d, $J = 8.0$ Hz; 6.59, 1H, dd, $J = 8.0$, 1.5 Hz and 6.39, 1H, d, $J = 1.5$ Hz could be assigned to H-5, H-6 and H-8 respectively (McPherson et al., 1983). The signal for two protons doublets ($J = 8.0$ Hz) at δ 8.01 was suggested for H-2′ and H-6′ and that at δ 6.92 for H-3′ and H-5′. These assignments were clearly supported from ^1H-^1H COSY and NOESY experiments. The long range coupled ($J = 1.8$ Hz) signals at δ 6.97 (1H) and 4.99 (2H) were assigned to H-9 and H_2-2 respectively. These signals confirmed the compound **2** as a derivative of 3-benzylidene-4-chromanone (Masterova et al., 1991). The position of the vinylic proton was indicative of a *cis* (Z) double bond at C_3–C_9 as the corresponding signal for a *trans* (*E*)-isomer (δ 7.60, 1H, t, $J = 1.8$ Hz) appears at downfield (Bohler and Tamm, 1967). The ^1H NMR spectrum also revealed the presence of a hydroxy (δ 10.12, br s) and a methoxy group (δ 3.82, 3H, s) in the molecule. The hydroxy group has been placed at C-7 and the methoxy group at C-4 on the basis of ^1H NMR spectrum along with mass spectrum which showed characteristic signals at m/z 146 and 136 due to retro-Diels–Alder cleavage of the molecule. The compound **2** was thus characterized as *cis* (*Z*)-7-hydroxy-3-(4-methoxybenzylidine)-chroman-4-one which is the *cis* (*Z*) isomer of bonducelline (**5**). This structure was further confirmed from its ^{13}C NMR (vide Experimental) and HMBC experimental data. The HMBC experiment clearly showed that the ketone carbonyl at C-4 (δ 181.7) was correlated to H-5 (δ 7.84), H_2-2 (δ 4.99) and H-9 (δ 6.97). The first proton (H-5) was related to two aromatic carbons, C-7 (δ 165.1) and C-8a (δ 164.01) linked with oxygen while H_2-2 were related to C-9 (δ 139.8) which again showed correlation with H-2′ and H-6′ (δ 8.01). The last two protons were related to the aromatic oxygenated carbon at C-4′ (δ 161.8) which showed correlation with the proton of methoxy group (δ 3.82). Thus the compound **2** was unambigueously established as the *cis* (*Z*)-isomer of bonducellin and named isobonducellin.

Compound **3** was obtained as yellow needles. This chalcone derivative was previously synthesised (Venturella

Table 1
Antibacterial activity of the compounds **1**–**3**[a]

Microorganism (Gram +ve)	Compound **1** (μg)		Compound **2** (μg)		Compound **3** (μg)		Control Pencillin G (30 μg/ml)
	30	100	30	100	30	100	
Staphylococcus aureus	7	9	8	11	7	9	12
Bacillus subtilis	7	10	9	11	6	8	15
Bacillus sphaericus	6	8	8	10	7	9	14
(Gram−ve)							Streptomycin (30 μg/ml)
Chromobacterium violaceum	7	9	9	13	7	9	24
Klebsiella aerogenes	7	9	–	–	6	8	23
Pseudomonas aeruginosa	8	9	–	–	7	8	24

[a] Inhibitory zone diameters are in mm.

Table 2
Antifungal activity of the compounds **1**–**3**[a]

Microorganism	Compound (μg)		Compound **2** (μg)		Compound **3**		Control Clotrimazole (100 μg/ml)
	100	150	100	150	100	150	
Aspergillus niger	8	11	7	10	7	9	22
Candida albicans	7	10	8	11	7	9	25
Rhizopus oryzae	–	–	–	–	–	–	24

[a] Inhibitory zones diameters are in mm.

and Bellino, 1960). However, as the spectral data of the compound were not reported so far these have been recorded here (vide Experimental).

Compound **4** was isolated as a white amorphous powder and compound **5** as an yellow crystalline solid. They were characterized as the known compounds 5,7-dimethoxyflavanone (Bick et al., 1972) and bonducellin (McPherson et al., 1983) respectively from their spectral (UV, IR, ^{1}H and ^{13}C NMR and MS) data and by comparison of the values with those reported earlier for the compounds.

As the plant *Caesalpinia pulcherrima* is used for the treatment of various infections the antimicrobial activity of the constituents **1**–**3** was evaluated (Table 1). All these compounds showed moderate to good antibacterial activity against the gram-positive organisms, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus sphaericus*. Particularly, compound **2** was found to be a good antibacterial substance with an inhibition zone dia. of 11 mm at 100 μg/ml test conc. The compounds **1** and **3** showed moderate activity against the gram-negative organisms, *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Chromobacterium violaceum*, while the compound **2** was inactive against the first two organisms and showed moderate activity against only *Chromobacterium violaceum*. The antifungal activity of all the compounds **1**–**3** (Table 2) was also moderate against the organisms, *Aspergillus niger* and *Candida albicans* and they were inactive against *Rhizopus oryzae*.

3. Experimental

3.1. General

Mps uncorr. The spectra were recorded with the following instruments: UV, GBC Cintra 10e spectrometer; IR, Perkin-Elmer Spectrum RX I FT-IR; NMR, Varian Gemini-200 MHz; EIMS: VG- Micromass 7070 H (70 eV). The optical rotations were measured with a JASCO DIP 360 Digital Polarimeter and CD spectra with JASCO J-715 CD spectropolarimeter. Column Chromatography was performed with silica gel (BDH, 100–200 mesh) and TLC with silica gel GF_{254}.

3.2. Plant material

The aerial parts of *Caesalpinia pulcherrima* were collected from Tirumala Hills in September, 2002 and identified botanically. A voucher specimen (No. CP-AP-1) is preserved in our laboratory and another voucher specimen (IICP-150902) in IICT herbarium.

3.3. Bacteria and fungi

Six bacterial organisms, *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus*(MTCC-96), *Pseudomonas aeruginosa* (MTCC-741), *Klebsiella aerogenes* (MTCC-39), *Bacillus sphaericus* (MTCC 511), *Chromobacterium violaceum* (MTCC 2656) and three fungal organisms, *Rhizopus*

oryzae (MTCC 262), *Aspergillus niger* (MTCC 281) and *Candida albicans* (MTCC 3017) were obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on Nutrient agar slants and were subcultured in petridishes prior to testing. Nutrient agar and potato dextrose agar (PDA) were procured from Himedia Laboratories, Mumbai.

3.4. Extraction and isolation

Air dried whole plant (3 kg) was powdered and Soxhleted with hexane (8 lit) for 120 h. The defatted plant material was subsequently Soxhleted with acetone. The extract was filtered and concentrated by rotary evaporator. The thick brown residue (32 g) was chromatographed over silica gel, the column being eluted with solvents of increasing polarity using hexane and EtOAc. The fractions eluted with 25% EtOAC in hexane afforded compound **3** (8 mg) which crystallized from MeOH to form yellow needles. A mixture of **2** and **5** (19 mg) was obtained when the column was eluted with 40% EtOAc in hexane. Subsequent elution of the column with 50% EtOAc in hexane and evaporation of the solvents yielded white amorphous powder of **4** (18 mg). The column was next eluted with 60% EtOAc in hexane to produce the compound **1** (11 mg) which crystallized from MeOH to colourless needles. The mixture of **2** and **5** obtained from the main column was rechromatographed. The first compound (10 mg) was eluted with 35% EtOAc in hexane while the second (7 mg) with 40% EtOAc in hexane. Both the compounds crystallized separately from acetone to afford yellow needles.

3.4.1. 5,7-Dimethoxy-3′,4′-methylenedioxy flavanone (*1*)

Colourless powder. UV λ_{max}^{MeOH} nm (log ϵ): 284 (3.7757), 208 (3.9674); IR (KBr) ν_{max}: 1673, 1621, 1575, 1500, 1450 cm^{-1}; $[\alpha]_D^{25}$ -8.23^0 (*c* 0.5, $CHCl_3$); CD (MeOH): $\Delta\epsilon_{285}$ −1.5, $\Delta\epsilon_{308}$ +0.8; ^1H NMR ($CDCl_3$): δ 6.95 (1H, *d*, *J* = 1.5 Hz, H-2′), 6.91 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-6′), 6.82 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.14 (1H, *d*, *J* = 1.5 Hz, H-8), 6.10 (1H, *d*, *J* = 1.5 Hz, H-6), 5.99 (2H, *s*,–OCH_2O–), 5.31 (1H, *dd*, *J* = 13.0, 3.0 Hz, H-2), 3.89 (3H, *s*,–OMe-5), 3.82 (3H, *s*,–OMe–7), 2.98 (1H, *dd*, *J* = 17.0, 13.0 Hz, H-3), 2.78 (1H, *dd*, *J* = 17.0, 3.0 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 189.2 (C-4), 165.9 (C-8a), 164.8 (C-7), 162.3(C-5), 148.0 (C-3′), 147.9 (C-4′), 132.6 (C-1′), 120.0 (C-6′), 108.4 (C-5′), 106.8 (C-2′), 105.9 (C-4a), 101.3 (–OCH_2O–), 93.5 (C-8), 93.2 (C-6), 79.1 (C-2), 56.1, 55.9 (2×–OMe), 45.5 (C-3); MS *m*/*z* (rel.int): 328 (M^+, 31), 208 (9), 180 (32), 148 (100), 109 (94). (Found: C, 65.78; H, 4.82.$C_{18}H_{16}O_8$; requires: C, 65.85; H, 4.87%).

3.4.2. Isobonducellin (*2*)

Yellow needles, m.p. 156–158^0; UV λ_{max}^{MeOH} nm (log ϵ): 353 (3.9722), 235 (3.8664), 208 (4.1539); IR (KBr) ν_{max}: 3441, 1628, 1580, 1455, 1418 cm^{-1}; ^1H NMR (acetone-d_6): δ 10.12 (1H, br s,–OH), 8.01 (2H, d, *J* = 8.0 Hz, H-2′, H-6′), 7.84 (1H, *d*, *J* = 8.0 Hz, H-5), 6.97 (1H, *t*, *J* = 1.8 Hz, H-9), 6.92 (2H, d, *J* = 8.0 Hz, H-3′, H-5′), 6.59 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-6), 6.39 (1H, *d*, *J* = 1.5 Hz, H-8), 4.99 (2H, *d*, *J* = 1.8 Hz, H_2-2), 3.82 (3H, s, –OMe); ^{13}C NMR (acetone-d_6): δ 181.7 (C-4), 164.2 (C-7), 163.1 (C-8a), 160.9 (C-4′), 138.9 (C-9), 133.1 (C-3′, C-6′), 129.6 (C-5), 127.7 (C-3), 127.3 (C-1′), 116.4 (C-4a), 113.2(C-3′, C′-5), 110.5(C-6), 102.3(C-8), 75.5 (C-2), 54.7 (–OMe); MS *m*/*z* (rel.int.): 282 (83), 254 (12), 160 (42), 137 (72). (Found: C,72.26 ; H, 4.93. $C_{17}H_{14}O_4$ requires: C, 72.34 ; H, 4.96%).

3.4.3. 2′-Hydroxy-2,3,4′6′-tetramethoxychalcone (*3*)

Yellow needles, m.p. 120–122^0; UV λ_{max}^{MeOH} nm (log ϵ): 343 (3.9298), 212 (4.1258); IR (KBr) ν_{max}: 3441, 1628, 1580, 1455 cm^{-1}; ^1H NMR ($CDCl_3$): δ 14.36 (1H, *br s*, -OH), 8.10 (1H, *d*, *J* = 16.0 Hz, H-β), 7.96 (1H, *d*, *J* = 16.0 Hz, H-α), 7.25 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-6), 7.10 (1H, *t*, *J* = 8.0 Hz, H-5), 6.96 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-4), 6.13 (1H, *d*, *J* = 1.5 Hz, H-5′) 5.98 (1H, *d*, *J* = 1.5 Hz, H-3′), 3.92, 3.91, 3.88, 3.85 (3H each, *s*, 4×–OMe); ^{13}C NMR ($CDCl_3$): δ 192.9 (>C=0), 168.4 (C-2), 166.1 (C-6′>), 162.5 (C-4′>), 153.2 (C-3), 148.8 (C-1′), 137.2 (C-2′), 129.7 (C-5), 128.9 (C-6), 124.1 (C-4), 119.7 (C-5′), 113.7 (C-3′), 106.4 (C-1′), 93.7 (C-β), 91.2 (C-α), 61.3, 55.9, 55.8, 55.6 (4×–OMe); MS *m*/*z* (rel. int): 344 (M^+, 16), 313 (100), 207 (33), 181 (47), 138 (17) (Found: C, 66.42; H,5.87. $C_{19}H_{20}O_6$; requires: C, 66.27; H, 5.81%).

3.5. Studies on antibacterial activity

The ready made nutrient agar medium (39 g) was suspended in distilled water (1000 ml) and heated to boiling until it dissolved completely. The medium and petridishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min.

Agar cup bioassay was employed for testing antibacterial activity of the compounds (Linday et al., 1962). The medium was poured into sterile petridishes under aseptic conditions in laminar flow chamber. When the medium in the plate solidify, 0.5 ml of 24h cultured of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving compound in DMSO and different concentrations (30 and 100 μg/ml) were made. After incubation, cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentrations of test solutions (30 and 100 μg/ml) were added. Controls were maintained with DMSO and pencillin G (for gram-positive) and streptomycin (for gram-negative) (each with 30 μg/ml). The treated materials and the controls were kept in an incubator at 37° for 24 h. Inhibition zones were measured and diameter was calculated in mm. Three replicates were maintained for each treatment.

3.6. Studies on antifungal activity

The method followed for antifungal bioassay is similar to that followed for antibacterial assay where in the medium is potato dextrose agar 39 g/l and the control is clotrimazole. Different concentrations (100 and 150 μg/ml) of test solutions were tested. controls were maintained with DMSO with clotrimazole (100 μg/ml). The treated and the controls were kept in an incubator at room temperature for 48 h. Inhibition zones were measured and diameter was calculated in mm. Three replicates were maintained for each treatment.

Acknowledgements

The authors thank CSIR, New Delhi for financial assistance.

References

Bick, I.R.C., Brown, R.B., Hillis, W.E., 1972. Three flavanones from leaves of *Eucalyptus sieberi*. Aust. J. Chem. 25, 449–451.

Bohler, P., Tamm, Ch., 1967. The homoisoflavanones, a new class of natural product. Isolation and structure of eucomin and eucomol. Tetrahedron Lett 3479–3483.

Che, C.T., McPherson, D.D., Cordell, G.A., Fong, H.H.S., 1986. Pulcherralpin, a new diterpene ester from *Caesalpinia pulcherrima*. J. Nat. Prod 49, 561–569.

Linday, M.E., 1962. Practical introduction to microbiology. E & F.N. Spon Ltd.

Iinuma, M., Yokoyama, J., Ohyama, M., Tanaka, T., Ruangrungsi, X., 1994. Eight phenolic compounds in roots of *Sophara exigua*. Phytochemistry 35, 785–789.

Masterova, I., Suchy, V., Uhrin, D., Ubik, K., Grancaiova, Z., Bobovnicky, B., 1991. Homoisoflavanones and other constituents from *Muscari racemosum*. Phytochemistry 30, 713–714.

McPherson, D.D., Cordell, G.A., Soejarto, D.D., Pizzuto, J.M., Fong, H.H.S., 1983. Peltogynoids and homoisoflavonoids from *Caesalpinia pulcherrima*. Phytochemistry 22, 2835–2838.

McPherson, D.D., Che, T.T., Cordell, G.A., Soejarto, D.D., Pezzuto, J.M., Fong, H.H.S., 1986. Diterpenoids from *Caesalpinia pulcherrima*. Phytochemistry 25, 167–170.

Namikoshi, M., Nakata, H., Saitoh, T., 1987. Homoisoflavonoids from *Caesalpinia sappan*. Phytochemistry 26, 1831–1833.

Passador, E.A.P., Da-Silva, M.F., Das-Fo, E.R., Fernandes, J.B., Vieire, P.C., Pirani, J.R., 1997. A pyranochalcone and a flavanone from *Neoraputia magnifica*. Phytochemistry 45, 1533–1537.

Patel, A.D., Freyer, A.J., Webb, R.L., Zuber, G., Reichwein, R., Bean, M.F., Faucettle, L., Johnson, R.K., 1997. Pulcherrimins A-D, novel diterpene dibenzoates from *Caelsalpinia pulcherrima* with selective activity against DNA repair-deficient yeast mutants. Tetrahedron 53, 1583–1592.

Ragasa, C.Y., Hofilena, J.G., Rideout, J.A., 2002. New furanoid diterpenes from *Caesalpinia pulcherrima*. J. Nat. Prod 65, 1107–1110.

Venturella, P., Bellino, A., 1960. Oxidation of 2-hydroxychalcones. The Algar—Flynn—Oxyamada reaction on chalcones with a methoxyl in position 6. Ann. Chim. (Rome) 50, 202–219.