Isoflavonoids from *Bolusanthus speciosus* (Bolus) Harms Leguminosae

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Seven isoflavonoids have been isolated from the seeds of *Bolusanthus speciosus* (Bolus) Harms. Five of these compounds were identified as the isoflavones genistein (**1**), biochanin A (**2**), orobol (**3**), pratensein (**4**) and 3′-O-methylorobol (**5**). The remaining two compounds were characterised as the novel isoflavone 3′-O-methylpratensein (**6**) and the new 3-hydroxyisoflavanone bolusanthin (**7**). Identification and characterisation was by means of spectroscopic techniques (UV, IR, MS and ^{1}H NMR). NOe irradiation of the C-2 proton of 3′-O-methylpratensein (**6**) was used to establish unequivocally the oxygenation pattern of the B-ring.

Introduction

Bolusanthus speciosus (Bolus) Harms, is the only species of this genus of the family Leguminosae (tribe Sophoreae). The plant, known as the 'South African Wisteria', is indigenous to southern Africa, where its wood, which is resistant to white-ant attack, is used for making furniture. Furthermore, it is grown throughout the world as an ornamental [1]. We have reported elsewhere the isolation of quinolizidine alkoloids from *B. speciosus* [2]. As the plant is a papilionate legume it seemed possible that it may also produce isoflavonoids [3] and in the present communication we report the isolation of seven isoflavonoids, two of which are novel structures, from the seeds.

Results and Discussion

Five of the isoflavonoids were characterised as the isoflavones genistein (**1**), biochanin A (**2**), orobol (**3**), pratensein (**4**) and 3′-O-methylorobol (**5**), by a comparison of their spectral (UV, IR, ^{1}H NMR) properties with literature values [4–7] and in some cases by use of nuclear Overhauser experiments (nOe) in the ^{1}H NMR. Two of the isoflavones, pratensein (**4**) and 3′-O-methylorobol (**5**) proved to be very difficult to separate. It appears from the literature that these two isomeric isoflavones have not been separated previously when they occur together in a plant [7, 8]. We were able to achieve their separation finally by means of isocratic HPLC over silica using hexane-*n*-butanol-1,2-dichloroethane (80–16–4) as eluant.

One of the novel compounds (**6**), had a molecular formula of $C_{17}H_{14}O_6$ (M^+, 314.0786, calc. 314.0790). Its UV absorption in MeOH at 263 nm and IR absorption at 1655 were consistent with the presence of the aromatic and C = O moieties respectively of an isoflavone. The nature of the A and B ring substituents was suggested by the EI mass fragmentation pattern. Major ions at *m/z* 300 and *m/z* 285 indicated loss of 2 methyl groups from the molecular ion, whilst the base peak at *m/z* 153 could be attributed to a dihydroxylated A-ring portion following retro-Diels Alder fragmentation in the typical manner of isoflavones [9]. The location of the A-ring hydroxyl groups was established as C-5 and C-7 by the UV bathochromic shifts observed upon addition of $AlCl_3$ and NaOAc to the methanolic spectrum. The ^{1}H NMR spectrum contained a singlet at δ 8.25, characteristic of the C-2 proton of an isoflavone and two aromatic meta coupled doublets at δ 6.41 and δ 6.32 which were assigned to the protons at C-8 and C-6 respectively [5]. The remaining signals in the spectrum were attributable to B-ring protons and substituents. Peaks at δ 7.30, δ 7.20 and δ 7.04 arose from an aromatic ABX system of protons and a sharp singlet at δ 3.90 which integrated for 6 protons confirmed the presence of 2 methoxyl groups. Three B-ring substitution patterns can account for these observations: 2′,4′-dimethoxy, 3′,4′-dimethoxy and 2′,5′-dimethoxy. We were able to differentiate between these possibilities by means of nOe measurements. Irradiation at δ 8.25, the C-2 proton gave enhancements of comparable intensity of two of the signals from the ABX system; the *meta* coupled pro-

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ton at δ 7.30 (11%) and the *ortho* and *meta* coupled proton at δ 7.20 (9%). These unique transannular dipolar couplings indicated that C-2′ and C-6′ were unsubstituted and allowed unequivocal location of the two methoxyl groups at C-3′ and C-4′. The novel compound was thus identified as 5,7-dihydroxy-3′,4′-dimethoxy-isoflavone (**6**) (3′-O-methylpratensein).

The second of the novel isoflavonoids (**7**), which we have called bolusanthin, was identified initially as a C-5, C-7-dihydroxylated isoflavanone from its UV spectrum in MeOH which underwent bathochromic shifts upon addition of $AlCl_3$ or NaOAc. IR spectroscopy indicated the presence of a chelated C=O. MS indicated a plausible molecular ion at *m/z* 318 and prominent fragment ions at *m/z* 300 ($M^+ - H_2O$) and at *m/z* 153. The latter ion would result from RDA fragmentation of a dihydroxylated A-ring. Additional peaks at *m/z* 151 and *m/z* 137 implied the existence of a mono-hydroxylated and mono-methoxylated B-ring [9]. In the ^1H NMR spectrum the characteristic complex system of signals [10] for the C-2a, C-2b and C-3 protons in an isoflavanone were replaced by two doublets centred at δ 5.07 and δ 4.65, each of which integrated for one proton. The magnitude of the coupling constant (12.3 Hz) suggested that the signals are due to geminal protons and the chemical shifts are consistent with those due to protons attached to carbons bearing oxygenated functions. Thus the doublets at δ 5.07 and δ 4.65 have been assigned to the methylene protons at C-2 and MS considerations indicate that C-3 bears an hydroxyl substituent. A C-3 hydroxylated isoflavanone, secondifloran, has been isolated previously from *Sophora secondiflora* D.C. [11]. ^1H NMR spectroscopy revealed that bolusanthin (**7**) possesses five aromatic protons. Two meta coupled signals at δ 6.00 and δ 5.96 were assigned to the C-8 and C-6 protons respectively. The remaining aromatic protons formed an ABX system and the spectrum additionally contained a singlet at δ 3.89 arising from the protons of a methoxyl group. Transannular nuclear Overhauser experiments proved to be fruitless for bolusanthin (**7**) and sensitivity problems could probably account for the lack of success. In this case, dipolar couplings are now shared by the protons at C-2, C-2′ and C-6′, including the extra proton arising from the saturation at the C-2/C-3 bond. This will decrease the already small nuclear Overhauser effects between the protons of the heterocyclic and B-rings. However the B-ring substitution pattern could be discerned by an nOe involving irradiation at the methoxyl signal at δ 3.89. This produced enhancement of one of the two AB protons of the ABX system and enabled characterisation of the novel compound as 3,5,7,3′-tetrahydroxy-4′-methoxyisoflavanone (**7**). This represents only the second report of the occurrence in nature of a 3-hydroxylated isoflavanone [11] and underlines the close taxonomic relationship between *Bolusanthus* and *Sophora*.

From our current knowledge [12] of isoflavonoid biosynthesis, the *B. speciosus* isoflavonoids can be related in a plausible metabolic grid (Fig. 1). Thus

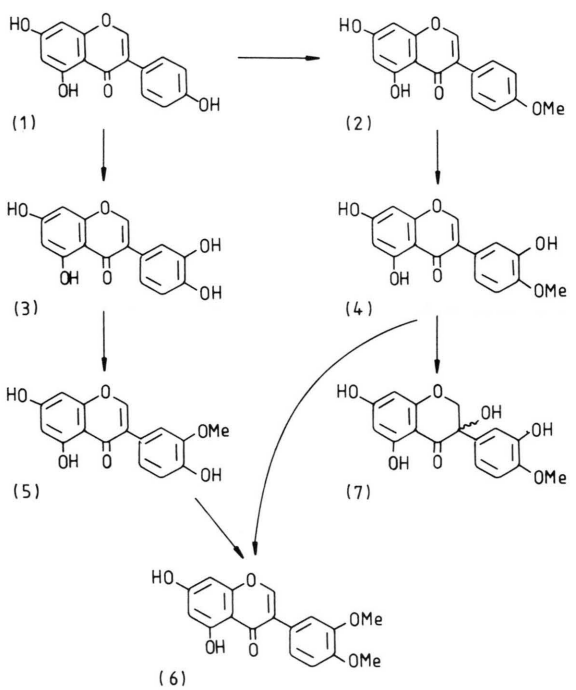

Fig. 1. Possible biogenetic relationships amongst *B. speciosus* isoflavonoids.

two routes could generate 3′-O-methylpratensein (**6**) from genistein (**1**). The first would involve methylation of the C-4′ hydroxyl to give **2** followed by hydroxylation at C-3′ to give **4** and its subsequent methylation. The alternative pathway would commence with hydroxylation at C-3′ **3** followed by sequential methylation of the two B-ring hydroxyls to give **5** and **6**. Bolusanthin (**7**) is formed presumably by hydration across C-2 and C-3 of pratensein (**4**).

Experimental

General techniques

UV and IR spectra were recorded on Perkin Elmer 402 and 298 instruments respectively. ^{1}H NMR spectra in acetone-D_6 were obtained using a Varian XL-300 instrument and chemical shifts reported are relative to TMS. MS were recorded on an Analytical ZAB-IF high resolution mass spectrometer operating at 70 eV.

Plant material and extraction of isoflavonoids

Seeds (200 g) of *B. speciosus*, purchased from Chiltern Seeds, U.K., were ground and defatted with petroleum ether (1L) for 48 hours by soxhlet extraction. The residue, after filtration was re-extracted with EtOH (0.5 L) by maceration. Concentration of the EtOH extract *in vacuo* at 40 °C gave a residue (6 g) which was suspended in 2% H_2SO_4 (aq) and extracted with $CHCl_3$ (4 × 150 ml). The acidic aqueous phase was separated and purified by acid-base extraction to yield a colourless oil (2.6 g) which contained alkaloids. The combined $CHCl_3$ phase was washed with 2% H_2SO_4 aq, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to a yellowish oil (950 mg).

Purification and characterisation of isoflavonoids

The yellowish oil was subjected to preparative TLC on Si GF_{254} using as solvents: i) $CHCl_3$ – MeOH (9–1), ii) Hexane-EtOAc – MeOH (60–40–10), iii) EtOAc-hexane (9–1) and iv) Hexane-*n*-BUOH (78–22). Isoflavonoids were detected using Fast Blue Salt B + NaOH (aq). HPLC was performed using an Altex isocratic liquid chromatograph (Model 110A) fitted with a Spherisorb-ODS 5 μm column (25 × 0.46 cm). Isoflavones (**4**) (Rt 3 mins) and **5** (Rt 4 mins) were eluted using a Hexane-BUOH-1,2-dichloroethane (80–16–4) mixture. Detection was by UV at 254 nm.

Genistein (**1**) Yield: 12 mg (0.006%); UV MeOH λmax nm: 262, 294(sh), 328(sh); MeOH + NaOAc λmax nm: 272, 309(sh), 379; MeOH + $AlCl_3$ λmax nm: 272, 325; MS *m/z* (rel. intens.): 270 (100)M^+, 153 (36), 152 (18), 135 (15), 124 (17), 118 (10); ^{1}H NMR 300 MHz acetone-D_6: δ 8.17 (1H, s, C-2), δ 7.44 (2H, d, J = 8.2 Hz, C-2′ and C-6′), δ 6.88 (2H, d, J = 8.2 Hz, C-3′ and C-5′), δ 6.39 (1H, d, J = 2.1 Hz, C-8), δ 6.24 (1H, d, J = 2.1 Hz, C-6).

Biochanin A (**2**) Yield: 6 mg (0.003%); UV MeOH λmax nm: 264, 330(sh); MeOH + NaOAc λmax nm: 264, 328(sh); MeOH + $AlCl_3$ λmax nm: 273, 310(sh), 375; MS *m/z* (rel. intens.); 284 (100) M^+, 270 (14), 153 (15), 133 (10), 132 (48), 124 (12), 118 (10); ^{1}H NMR 300 MHz acetone-D_6: δ 8.23 (1H, s, C-2), δ 7.59 (2H, d, J = 7.9 Hz, C-2′ and C-6′), δ 7.04 (2H, d, J = 7.9 Hz, C-3′ and C-5′), δ 6.45 (1H, d, J = 2 Hz, C-8), δ 6.33 (1H, d, J = 2 Hz, C-6), δ, 3.89 (3H, s, OMe).

Orobol (**3**) Yield: 4 mg (0.002%); UV MeOH λmax nm: 262, 293(sh), 328(sh); MeOH + NaOAc λmax nm: 271, 321; MeOH + $AlCl_3$ λmax nm: 271, 291(sh), 361(sh); MS *m/z* (rel. intens.): 286 (100) M^+, 229 (9), 153 (24), 134 (30), 124 (8), 120 (15); ^{1}H NMR 300 MHz acetone-D_6: δ 8.13 (1H, s, C-2), δ 7.14 (1H, d, J = 2.1 Hz, C-2′), δ 6.93 (1H, dd, J = 8.2 Hz, 2 Hz, C-6′), δ 6.87 (1H, d, J = 8.2 Hz, C-5′), δ 6.41 (1H, d, J = 2 Hz, C-8), δ 6.28 (1H, d, J = 2 Hz, C-6).

Pratensein (**4**) Yield: 105 mg (0.053%) UV MeOH λmax nm: 262, 294, 330(sh); MeOH + NaOAc λmax nm: 271, 325(sh); MeOH + $AlCl_3$ λmax nm: 272, 310(sh), 371; MS *m/z* (rel. intens.) 300 (100) M^+, 285 (20), 272 (16), 257 (10), 229 (17), 153 (50), 148 (20), 131 (17), 120 (15); ^{1}H NMR 300 MHz acetone-D_6: δ 8.17 (1H, s, C-2), δ 7.14 (1H, d, J = 2 Hz, C-2′), δ 7.06 (1H, dd, J = 8.4 Hz, 2.1 Hz, C-6′), δ 6.99 (1H, d, J = 8.4 Hz, C-5′), δ 6.42 (1H, d, J = 2 Hz, C-8), δ 6.29 (1H, d, J = 2 Hz, C-6), δ 3.86 (3H, s, OMe).

3′-O-Methylorobol (**5**) Yield: 65 mg (0.033%), UV MeOH λmax nm: 263, 294(sh), 340(sh); MeOH + NaOAc λmax nm: 274, 330; MeOH + $AlCl_3$ λmax nm: 273, 311; MS *m/z* (rel. intens.): 300 (100) M^+, 285 (15), 271 (10), 268 (36), 257 (11), 229 (15), 153 (45), 148 (18), 131 (21), 120 (14); ^{1}H NMR 300 MHz acetone-D_6: δ 8.20 (1H, s, C-2), δ 7.26 (d, J = 2 Hz, C-2′), δ 7.07 (1H, dd, J = 8.1 Hz, 2 Hz, C-6′), δ 6.89 (1H, d, J = 8.1 Hz, C-5′), δ 6.43 (1H, d, J = 2.2 Hz, C-8), δ 6.29 (1H, d, J = 2.2 Hz, C-6), δ 3.89 (3H, s, OMe).

3′-O-Methylpratensein (**6**) Yield: 4 mg (0.002%); UV MeOH λmax nm: 263, 295(sh); MeOH + NaOAc λmax nm: 274, 330; MeOH + $AlCl_3$ λmax nm: 275, 309(sh), 375; IR $CHCl_3$ υmax cm^{-1}: 3350 (OH), 1655 (chelated C=O), 1600–1500 (aromatics); MS: *m/z* (rel. intens.): 314.0786 (56) M^+ (calc. for $C_{17}H_{14}O_6$: 314.0790), 300 (15), 285 (100), 272

(15), 270 (16), 153 (20), 150 (11), 133 (13), 132 (46); ^{1}H NMR 300 MHz acetone-D_6: δ 8.25 (1H, s, C-2), δ 7.30 (1H, d, J = 1.9 Hz, C-2′), δ 7.20 (1H, dd, J = 8.2 Hz, 1.9 Hz, C-6′), δ 7.04 (1H, d, J = 8.2 Hz, C-5′), δ 6.46 (1H, d, J = 2 Hz, C-8), δ 6.32 (1H, d, J = 2 Hz, C-6), δ 3.90 (3H, s, OMe).

Bolusanthin (**7**) Yield: 3 mg (0.001%) UV MeOH λmax nm: 231(sh), 290, 318(sh); MeOH + NaOAc λmax nm: 247, 327(sh); MeOH + $AlCl_3$ λmax nm: 271(sh), 315, 382(sh); IR $CHCl_3$ υmax cm^{-1}: 3400–3300 (OH), 1660 (chelated C=O), 1630–1500 (aromatics); MS m/z (rel. intens.): 318.0743 (31) M^+ (calc. for $C_{16}H_{14}O_7$ 318.0740), 300 (26), 289 (20), 270 (19), 166 (44), 165 (26), 164 (38), 153 (100), 151 (14), 137 (38), 128 (14), 123 (10); ^{1}H NMR 300 MHz acetone-D_6: δ 7.23 (1H, d, J = 1.9 Hz, C-2′), δ 7.04 (1H, dd, J = 8.0 Hz, 1.9 Hz, C-6′), δ 6.87 (1H, d, J = 8.0 Hz, C-5′), δ 6.00 (1H, d, J = 2.2 Hz, C-8), δ 5.96 (1H, d, J = 2.2 Hz, C-6), δ 5.07 (1H, d, J = 12.3 Hz, C-2a), δ 4.65 (1H, d, J = 12.3 Hz, C-2b), δ 3.89 (3H, s, OMe).

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