Anthraquinones from the bark of Senna macranthera

ALEXSANDRO BRANCO¹, ANGELO C. PINTO², JAN SCHRIPSEMA³ and RAIMUNDO BRAZ-FILHO⁴

¹Laboratório de Fitoquímica, Departamento de Saúde, Universidade Estadual de Feira de Santana, Av. Transnordestina, s/n, Bairro Novo Horizonte, 44036-900 Feira de Santana, BA, Brasil
²Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia, Bloco A, Cidade Universitária, Ilha do Fundão, 21945-970 Rio de Janeiro, RJ, Brasil
³Grupo Metabolômica, Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000, Parque Califórnia, 28015-620 Campos dos Goytacazes, RJ, Brasil
⁴Setor de Química de Produtos Naturais, LCQUI-CCT, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000, Parque Califórnia, 28015-620 Campos dos Goytacazes, RJ, Brasil

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ABSTRACT

2-acetyl physcion (2-acetyl-1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthraquinone, 2), a rare anthraquinone, was isolated from Senna macranthera var. nervosa (Vogel) H.S. Irwin & Barneby (Fabaceae). The chemical structure was elucidated and all 1H and 13C NMR chemical shifts were assigned by NMR one- (1HNMR, {1H}13CNMR, and APT-13CNMR) and two (COSY, NOESY, HMQC and HMBC) dimensional of this natural compound. Furthermore, the minor anthraquinones chrysophanol (3), chrysophanol-8-methyl ether (4) and physcion (5) were characterized by GC-MS analysis. The occurrence of the anthraquinones 3-5 confirms that S. macranthera is a typical representative of the genus Senna.

Key words: anthraquinone, 2-acetyl-physcion, Fabaceae, Senna macranthera.

INTRODUCTION

The genus Senna is known to produce various classes of aromatic compounds, e.g. quinones, normal (Barba et al. 1992) and dimeric anthraquinones (Koyama et al. 2001), naphthopyrones (Barbosa et al. 2004) and flavonoids (Baez et al. 1999). S. macranthera var. nervosa (Vogel) H.S. Irwin & Barneby (syn. Cassia macranthera), commonly known as “alleluia” (subfamily Papilionoidae, family Fabaceae), is a 6-8 m high tree with a trunk of up to 30 cm of diameter. The leaves are composed of two pairs of opposing leaflets. In Brazil, the tree is found from Ceará in the North down to São Paulo in the South. It is used mainly as an ornamental plant in cities because of lush yellow flowers and rapid growth (Lorenzi 2000). Previous phytochemical studies on the bark of S. macranthera collected in Belo Horizonte, Brazil, revealed the presence of rubrofusarin (1), 6-O-galactosylnrubrofusarin, 3,5-dihydroxy-8-isobutenyl-2-methyl-7-methoxychromone and β-sitosterol (Oliveira et al. 1977). A galactomannan with anticoagulant activity has been isolated from the endosperm of seeds (Pires et al. 2001).

The anthraquinones are traditionally used as pigments and medicines, and are widely distributed in nature, occurring in both free and glycosidic form (Brune-ton 1991). Several analytical methods have been used to analyse these compounds, including gas chromatography coupled to mass spectrometry (GC-MS) (Waterman and Mole 1994). This method permits the analysis of non-glycosylated anthraquinones with (Zuo et al. 2008) or without derivatisation (Mueller et al. 1999, Liu et al. 2007).
In this paper we report the identification of 2-acetyl physcion (2) by spectrometric methods, together with the characterisation of others minor anthraquinones (3-5) (Fig. 1) using GC-MS analysis from S. macranthera.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

NMR spectra were recorded in CDCl₃ solution at 400 MHz for ¹H and 100 MHz for ¹³C on a JEOL Eclipse+ 400 spectrometer. Chemical shift values are reported relative to TMS, which was either used as internal standard or by reference to solvent signals: CHCl₃/CDCl₃ (δ H 7.26 and δ C 77.00). GC analyses were recorded on a Hewlett Packard model 5790 A gas chromatograph using glass capillary column (11 m × 0.25 μm) coated with SE-54 (df = 0.25 μm). GC-MS spectra were run at 70 eV on a Shimadzu QP-2000 spectrometer. The data were collected on an HP 3396-II integrator. TLC: silica gel (Merck, Kieselgel 60), spots visualised by UV (254 and 360 nm) and exposure to I₂ vapour. TLC was used for analysis of fractions collected from CC.

PLANT MATERIAL

The bark of S. macranthera was collected in Campinas, São Paulo State, Brazil, in September 2000. The identification was performed by Marcia Dias Campos, and a voucher specimen (n. 89281) is deposited at the Herbarium of the State University of Feira de Santana, Brazil.

EXTRACTION AND ISOLATION

The bark (500 g) of S. macranthera was dried at controlled temperature (60 °C). After drying, the bark was shed and extracted with n-hexane (2 L) and dichloromethane (2 L) at room temperature for seven days each, successively. During the evaporation of the hexane under reduced pressure in the rotary evaporator, the formation of a red coloured precipitate was observed, filtered and identified as rubrofusarin 1 (2.0 g) (Branco et al. 2008). The residue, after further drying, yielded the respective hexane extract (9.1 g) that was chromatographed with a gradient of EtOAc in hexane to furnish an additional quantity of 1 (0.8 g). The dichloromethane extract (52.0 g) was also chromatographed on a silica gel column (65.3 g) in the same manner to yield a total of 10 fractions of ca. 100 mL each that were collected and combined on the basis of TLC comparison. Fraction 3 eluted with hexane/EtOAc (3:1), furnished 2 as red crystals (6.5 mg). The fractions 8-9 eluted with EtOAc/EtOH (1:1), were united and analysed by GC-MS (Fig. 2).

2-ACETYL PHYSICION 2

Red solid; IR (KBr) νmax cm⁻¹: 3442, 1700, 1691, 1615, 1598, 1474, 1443, 1208, 1235, 1165; ¹H [400 MHz, CDCl₃]: δ H 7.65 (s, H-4), 7.38 (d, J = 2.6 Hz, H-5), 6.71 (d, J = 2.6 Hz, H-7), 3.95 (s, Me-O-6), 2.61 (s, 3H-12), 2.39 (s, 3H-13), 12.48 (s, HO-1), 12.18 (s, HO-8). ¹³C NMR [75 MHz, CDCl₃]: δ C 159.28 (C-1), 136.30 (C-2), 144.59 (C-3), 135.03 (C-4a), 166.88 (C-6), 165.42 (C-8), 110.15 (C-8a), 190.65 (C-9), 114.09 (C-9a), 181.46 (C-10), 133.01 (C-10a), 203.03 (C-11), 122.08 (C-4), 108.75 (C-5), 106.86 (C-7), 56.17 (MeO-6), 31.85 (C-12), 20.13 (C-13). GC-MS (70 eV) m/z (rel. int.): 326 ([M-H]⁺*, 68%), 311 (M-Me⁺*, 100), 283 (M-H₂O, 15), 298 (M-CO, 1), 283 (m/z 311-CO, 3), 255 (m/z 283-CO, 2), 227 (m/z 255-CO, 9).

RESULTS AND DISCUSSION

The IR spectrum of compound 2 showed bands for two hydroxyl groups (νmax 3442 cm⁻¹), three carbonyl groups (νmax 1700, 1615 and 1598 cm⁻¹) and aromatic rings (νmax 1550 and 1474 cm⁻¹). The ¹H NMR spectrum revealed signals corresponding to the presence of two peri-positioned chelated hydroxyl groups (δ H 12.48, HO-1, and 12.18, HO-8) (Schripsema and Dagnino 1996), three aromatic hydrogens [one singlet at δ H 7.65 (H-4) and two doublets compatible with two meta-coupled hydrogen atoms at δ H 7.38 (d, J = 2.6 Hz) and 6.71 (d, J = 2.6 Hz)], one methoxyl function (δ H 3.95) and two methyl groups linked to sp³ carbon atoms [δ H 2.61 (s, 3H-12) and 2.39 (s, 3H-13)]. These data and comparative analysis of ¹H- and APT-¹³C NMR
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Fig. 1 – Structures of identified compounds in S. macranthera.

spectra [twelve sp$^2$ quaternary carbon atoms, including three carbonyl groups at $\delta C$ 203.03 (C-11), 190.65 (C-9) and 181.46 (C-10), three sp$^2$ methines and three methyl groups at $\delta C$ 56.17 (CH$_3$O), 31.85 (CH$_3$-12, acetyl function) and 20.13 (CH$_3$-13)], together with the molecular ion in the mass spectrum at $m/z$ 326 ([M]$^+$, 58%), suggested the structure of this physcion derivative. Heteronuclear 2D shift-correlated NMR techniques $^1$H-$^1$C-COSY (HMOC) and $^1$H-$^1$C-COSY-3JCH (HMBIC) were used for the correct $^1$H and $^13$C NMR chemical shift assignments of anthraquinone 2. The location of the acetyl group at carbon atom C-2 was confirmed by heteronuclear long-range couplings C-2 ($\delta C$ 136.30) and HO-1 ($\delta H$ 12.48, $^3$JCH), H-4 ($\delta H$ 7.65, $^3$JCH) and both 3H-12 ($\delta H$ 2.61, $^3$JCH) and 3H-13 ($\delta H$ 2.39, $^3$JCH). The $^1$H-$^1$H-NOESY spectrum further confirmed the structure, showing interactions between 3H-13 and H-4, and between the methoxyl group and both H-5 and H-7.

Thus, the structure of 2 was established as 1,8-dihydroxy-2-acetyl-3-methyl-6-methoxy-9,10-anthraquinone (2-acetyl physcion). This is the first report of the anthraquinone 2 in a Senna species. All $^1$H and $^13$C chemical shifts were unambiguously assigned. This compound has been reported only once in the literature (Wei et al. 2007), and the attributions to the carbons C-4a ($\delta C$ 135.03) and C-10a ($\delta C$ 133.01) were corrected.

The fractions of the dichloromethane extract obtained by SiO$_2$ fractionation were analysed by GC-MS to detect other polyketid. Only the fraction 8-9 showed the presence of peaks the typical fragmentation pattern of anthraquinones: intense molecular ion, loss of CO (28 Da) and other fragmentations with weak intensity (Song et al. 2009). Figure 2 and Table I show the chromatogram and mass spectra of this fraction, respectively.

The peaks corresponding to Tr at 14.6, 16.1 and 17.3 min showed molecular ion base peaks ([M]$^+$, 100%) at $m/z$ 254, 268 and 284, and loss of CO to furnish peaks at $m/z$ 226 (13%), 240 (4%) and 256 (5%), respectively. The molecular mass of 4 ([M]$^+$, $m/z$ 268) is 14 Da higher than 3 ([M]$^+$, $m/z$ 254), indicating the presence of an additional methylene unit. This -CH$_2$- unit was attributed to formation of one methoxyl group at C-8 (C-OH to C-OCH$_3$).

Thus, the GC-MS analyses permitted the identification of chrysophanol (3), chrysophanol-8-methyl ether

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TABLE I

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<th>Structure</th>
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</table>

*Retention time in minute.

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REFERENCES


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