Pergamon

0031-9422(94)00641-5

Phytochemistry, Vol. 38, No. 3, pp. 729–731, 1995

0031-9422/95 $9.50 + 0.00

A XANTHONE *C*-GLYCOSIDE FROM *IRIS NIGRICANS*

SULEIMAN AL-KHALIL,* HIDEKI TOSA† and MUNEKAZU IINUMA†

Faculty of Pharmacy, Jordan University for Women, P.O. Box 961343, Amman, Jordan; †Gifu Pharmaceutical University, Gifu 502, Japan

(*Received in revised form* 4 *July* 1994)

Key Word Index—*Iris nigricans*; Iridaceae; rhizomes; *C*-glycosides; 2-β-D-glucopyranosyl-1,3,5,8-tetrahydroxyxanthone.

Abstract—The isolation and identification of seven xanthones from an extract of the rhizomes of *Iris nigricans* is described. The isolated compounds are the xanthones, 1,3,6,7-tetrahydroxy-1,6,8-trihydroxy-2-methoxy-1,6-dihydroxy-3,7-dimethoxy- and 1,3,5,8-tetrahydroxy-, and the xanthone *C*-glycosides mangiferin, 7-*O*-methylmangiferin and a new compound 2-β-D-glucopyranosyl-1,3,5,8-tetrahydroxyxanthone (nigricanside). The new structure was established by detailed spectral and chemical analysis.

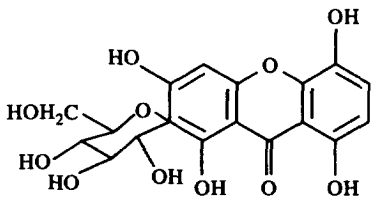
INTRODUCTION

Iris nigricans is a perennial herb endemic in the fallow fields of Jordan [1, 2]. Extracts of this species have been shown to contain isoflavones and led to the isolation and characterization of two new isoflavones, i.e. nigricin and nigricanin, respectively [3]. As part of our continuing studies, this paper reports the isolation and identification of seven xanthones from an extract of the rhizomes of *I. nigricans*. These xanthones include the known ones 1,3,6,7-tetrahydroxyxanthone (norathriol) **1**, 1,6,8-trihydroxy-2-methoxy-xanthone (bellidifolin) **2**, 1,6-dihydroxy-3,7-dimethoxyxanthone **3**, mangiferin **4**, 1,3,5,8-tetrahydroxy-xanthone (desmethylbellidifolin) **5**, 7-*O*-methylmangiferin **6** and a new xanthone *C*-glycoside, 2-β-D-glucopyranosyl-1,3,5,8-tetrahydroxyxanthone **7**, which was assigned the trivial name, nigricanside.

RESULTS AND DISCUSSION

An ethanol extract of the rhizome was partitioned between water and chloroform and *n*-butanol, respectively. The chloroform phase after drying was subjected to silica gel chromatography to give the known compounds **1–3**. Compound **3**, 1,6-dihydroxy-3,7-dimethoxyxanthone, was obtained as yellow needles. This is the first report of the isolation this compound from an *Iris* sp. However, this compound, **3**, has been obtained by methylation of norathriol 6-*O*-β-D-glucoside (tripteroside) followed by hydrolysis with 5% H_2SO_4 [4]. The *n*-butanol phase was subjected to Sephadex gel chromatography to give the known compounds **4–6** and the new xanthone *C*-glycosides **7**.

The UV spectrum of **7** (231, 255, 278, and 338 nm) was similar to that of 1,3,5,8-tetraoxygenated xanthone [5–8], but different from that of 1,3,5,6-tetraoxygenated ones [9, 10]. The spectrum exhibited a bathochromic shift with $AlCl_3$ indicating the presence of chelated hydroxyl group(s) at position 1 and/or 8 [7, 10]. Because the reaction was not reversed upon addition of HCl, the *peri* position(s) [1 and/or 8] must be OH-substituted [11]. A bathochromic shift with NaOAc was also observed, indicating a free hydroxyl group at position 3 and/or 6 [7, 10], while the maxima were unaffected by $NaOAc/H_3BO_3$ indicating the absence of *ortho*-hydroxyl groups [10]. In addition, a 1,3-dihydroxy system must be in the A ring of this xanthone, as the UV spectra in the presence of NaOMe and NaOAc are not superimposable [10]. The IR spectrum indicated the presence of hydroxyl (3395 cm^{-1}) and α,β-unsaturated ketone (1644 cm^{-1}) [5]. ^1H NMR analysis of **7** suggested the presence of two chelated hydroxyl protons (δ 12.85 and 13.1), which disappeared upon the addition of D_2O, three aromatic protons, amongst which one appeared as a singlet at δ 6.42 and could be assigned to the C-4 proton, since the C-2 proton appeared at a higher field than that of C-4 [12, 13]. The remaining two aromatic protons appeared as *ortho*-split



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*Author to whom correspondence should be addressed.

doublets, centred at δ7.21 and 6.86 (J_9 = 8.89 Hz) which were assignable to the C-6 and C-7 hydrogens of the xanthone nucleus [12, 14, 15]. A broad signal integrating for six protons at δ3.2–4.15 and a doublet (J = 9.1 Hz) at δ4.69 were assigned to the aliphatic protons and the anomeric proton of the β-linked residue sugar moiety [16]. Compound **7** gave a positive Gibbs test, indicating that an aromatic proton is located *para* to a phenolic group [10]. The site of the sugar linkage to the aglycone in **7** was considered to be at C-2 of 1,3,5,8-tetrahydroxyxanthone, since in the ^{13}C NMR spectrum a signal appeared at δ107.8, meanwhile in the aglycone the compounding signal appeared at δ98.2 [7, 11]. The hexose substituent at C-2 gave a pattern of ^{13}C NMR signals similar to that exhibited by the *C*-linked glucose in mangiferin [17, 18]. Compound **7** resisted the usual acid hydrolysis, but refluxing with HI gave 1,3,5,8-tetrahydroxyxanthone [14], which was identical to the isolated compound **5**. Compound **7**, $C_{19}H_{18}O_{11}$, gave a molecular ion at m/z 404 $[M - 18]^+$ with a fragment ion (base peak) at m/z 273 $[M - C_5H_9O_5]^+$, indicating that the glucose is *ortho* to a chelated hydroxyl group [19]. Further confirmation was made by direct comparison with the spectral data of **5**. Thus, the structure of **7** was determined as 2-β-D-glucopyranosyl-1,3,5,8-tetrahydroxy-9H-xanthene-9-one, which was given the trivial name, nigricanside.

EXPERIMENTAL

General. Mps: uncorr. UV were obtained in MeOH, IR in KBr pellets. ^{1}H NMR at 200 MHz and ^{13}C NMR at 100 MHz with TMS as int. standard; chemical shifts are reported in δ units. Silica gel (Kiesel gel 60, Merck) was used for CC, (Kieselgel 60F_{254} Merck) used for TLC. Sephadex LH-20 was used for gel chromatography. Na_2SO_4 was routinely used for drying solvents and all solvents were evapd under red. pres. at 40°.

Plant material. Iris nigricans Dinsm. was collected in the vicinity of Madaba, 40 km south of Amman, Jordan, in April 1990 and identified by Prof. D. Al-Eisawi, Plant Taxonomist, Department of Biological Sciences, Faculty of Science. A herbarium specimen is deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Jordan, Amman-Jordan.

Extraction and fractionation. Air-dried and chopped rhizomes (6.4 kg) were extracted by percolation with EtOH (28 l). After the solvent was evapd, a syrupy residue (816 g) was obtained. This was suspended in H_2O (1.5 l) and extracted with $CHCl_3$ (1 l × 3) [fr. A, 116.3 g] and with *n*-BuOH (1 l × 3) [fr. B, 178.1 g], respectively.

Chromatography of fr. A. Fr. A was chromatographed over silica gel (350 g) and eluted with varying proportions of petrol, $CHCl_3$ and MeOH. Elution with $CHCl_3$–MeOH (3:2) (1.5 l) afforded a residue (24.3 mg), which was recrystallized from MeOH to give 1,3,6,7-tetrahydroxyxanthone (norathriol) **1** (15.6 mg), identical by direct comparison with spectral and lit. data (UV, IR, MS, ^{1}H NMR) [20, 21]. Continued elution with $CHCl_3$–MeOH (2:3) yielded a solid residue (43.6 mg) which showed two major spots on TLC. Prep. TLC (EtOAc–MeOH–H_2O, 20:3:2) gave 2 pure compounds **2** and **3**, respectively. Compound **2**, mp 259–260° (MeOH) was identified as 1,6,8-trihydroxy-2-methoxyxanthone (bellidifolin) by direct comparison with lit. data (UV, ^{1}H NMR, MS, mp) [22, 23]. Compound **3**, mp 266–267° (MeOH) was identified as 1,6-dihydroxy-3,7-dimethoxyxanthone by direct comparison with lit. data (UV, ^{1}H NMR, MS, mp) [4] and conversion to its diacetate [4].

Chromotagraphy of fr. B. Fr. B was chromatographed over Sephadex LH-20 (350 g) using MeOH–H_2O (9:1) as eluent; 40 frs were collected (50 ml each) and combined according to TLC analysis. Frs 1–12 gave mangiferin **4** (78 mg), mp 271° (MeOH) identical by direct comparison with spectral and lit. data (UV, ^{1}H, ^{13}C NMR, MS, mp) [8, 21, 24]. Frs 13–18 yielded 1,3,5,8-tetrahydroxyxanthone **5** (14.1 mg), mp 292° (MeOH) identical by direct comparison with spectral and lit. data (UV, ^{1}H, ^{13}C NMR, MS, mp) [7, 8, 25]. Frs 19–25 gave 7-*O*-methylmangiferin **6** (23.6 mg), mp 239–240°, $[\alpha]_D^{23}$ + 31.3° (pyridine; *c* 0.5) identical by direct comparison with lit. data ($[\alpha]_D$, UV, ^{1}H NMR, MS, mp) [12]. Frs 26–40 yielded a mixt. (134.4 mg), which showed 1 major spot on TLC and was subjected to silica gel CC (30 g). Elution with $CHCl_3$–MeOH (13:7) [2.5 l] yielded a solid residue (98.6 mg) which recrystallized from MeOH to furnish **7** (72.3 mg).

2-β-D-*Glucopyranosyl*-1,3,5,8-*tetrahydroxy*-9H-*xanthene*-9-*one* (*Nigricanside*) **7**. Pale yellow needles, mp 264–266° $[\alpha]_D^{23}$ + 24.6° (pyridine; *c* 0.4). Gibbs test (+). Analyt. calcd. $C_{19}H_{18}O_{11}$: C, 54.03, H, 4.30. Found: C, 53.78, H, 4.23. EIMS m/z (rel. int.): 404 (15), 386 (9), 368 (35), 350 (12), 274 (56), 273 (100). UV λ_{max} nm: 231, 255, 278, 338; + $AlCl_3$ 226 (sh), 263, 289, 326, 376; + $AlCl_3$–HCl 226 (sh), 257, 291, 328, 376; + NaOMe 235, 260, 304, 360; + NaOAc 248 (sh), 272, 361; + NaOAc–H_3BO_3 252, 276, 338. IR ν_{max} cm^{-1}: 3395 (OH), 1644 (C=O, con.), 1618, 1595, 1505, 1320, 1073, 1040, 1025, 855. ^{1}H NMR (DMSO-d_6): δ3.2–4.15 (6H, *br*, al. H), 4.69 (1H, *d*, J = 8.89 Hz, H-1′), 6.42 (1H, *s*, H-4), 7.21 (1H, *d*, J = 8.89 Hz, H-6), 6.86 (1H, *d*, J = 8.89 Hz, H-7), 12.85 (1H, *s*, OH-8, exchangeable with D_2O), 13.1 (1H, *s*, OH-1, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6): δ161.6 (C-1), 107.8 (C-2), 163.6 (C-3), 93.2 (C-4), 156.1 (C-4a), 135.2 (C-5), 124.2 (C-6), 111.6 (C-7), 150.6 (C-8), 107.4 (C-8a), 179.9 (C-9), 101.2 (C-9a), 143.5 (C-10a), 72.9 (C-1′), 70.4 (C-2′ or C-4′), 78.8 (C-3′), 70.1 (C-4′ or C-2′), 81.4 (C-5′), 61.9 (C-6′).

Octaacetate of **7**. A soln of **7** (20 mg) in dry pyridine and Ac_2O was heated at 100° for 4 hr. The reaction mixt. was poured into ice–H_2O and the ppt. collected, washed with H_2O, dried and recrystallized from MeOH as needles, mp 205–207°. Analyt. calcd. for $C_{35}H_{34}O_{19}$: C, 55.41, H, 4.52. Found C, 55.18, H, 4.53. ^{1}H NMR (DMSO-d_6): δ1.78 (3H, *s*, al. OAc.), 2.01 (3H, *s*, al. OAc), 2.03 (3H, *s*, al. OAc), 2.05 (3H, *s*, al. OAc), 2.47 (3H, *s*, ar. OAc), 2.55 (9H, *s*, 3 × ar. OAc), 4.81 (1H, *d*, J = 9.1 Hz, H-1′), 5.71 (1H, *s*, H-4), 7.53 (1H, *d*, J = 8.6 Hz, H-6), 7.06 (1H, *d*, J = 8.6 Hz, H-7).

Treatment of **7** *with* HI. A soln of **7** (20 mg) in phenol (0.2 mg) was refluxed with HI (*d*, 1.7, 1 ml) for 4 hr. The reaction mixt. was poured into 5% $NaHSO_3$ soln (20 ml), the ppt. collected, washed with H_2O and dried. The product obtained was identical by direct comparison (UV, IR, co-TLC, m.mp) with **5**. The product was further heated with Ac_2O and NaOAc for 1 hr and was pptd by adding H_2O, collected on filter paper, washed with H_2O and recrystallized from MeOH to give the tetraacetate of **5** as needles, mp 241°. ^1H NMR ($CDCl_3$): δ2.2–2.34, 2.45 (*s*, ar, OAc), 6.91 (1H, *d*, J = 2.18 Hz, H-2), 7.12 (1H, *d*, J = 2.18 Hz, H-4), 7.23 (1H, *d*, J = 9.1 Hz, H-6), 7.54 (1H, *d*, J = 9.1 Hz, H-7).

Ferric chloride oxidation of **7**. Compound **7** (15 mg) was refluxed with an aq. soln of $FeCl_3$ (0.5 g in 5 ml H_2O) for 6 hr. After cooling the reaction mixt. was filtered and the filtrate passed through a column of Amberlite IR-120 (H^+ form) and IR-4B (OH^- form) and evapd to a syrupy residue, which was examined by PC. R_f0.18 (brown, glucose); 0.21 (reddish brown, arabinose) (*n*-BuOH–HOAc–H_2O, 4:1:2). Colour reaction with 0.1 N aniline hydrogen phthalate [14].

Acknowledgements—The authors are grateful to Professor Sun Hong-Fa, Northwest Institute of Biology Academic Sinica, Xining, Qinghai, China for spectra of 1,3,5,8-tetrahydroxyxanthone, and to Dr G. Kitov, Department of Pharmacognosy, Faculty of Pharmacy, Sofia, Bulgaria for spectra of norathriol and mangiferin.

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