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NEW 1,3,5-TRIOXYGENATED XANTHONES IN *CANSCORA DECUSSATA**

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Key Word Index—*Canscora decussata*; Gentianaceae; trioxygenated xanthone; 1-methoxy-3,5-dihydroxyxanthone; xanthone-*O*-glycoside; 1-methoxy-5-hydroxyxanthone-3-*O*-rutinoside.

Abstract—Aerial parts of *Canscora decussata* have been shown to contain 1-methoxy-3,5-dihydroxyxanthone and its 3-*O*-rutinosyl derivative. The identity of these compounds was established by chemical transformation and spectral (UV, IR, PMR, MS) evidence of the compounds and their derivatives. This is the first demonstration of the occurrence of the two compounds in nature.

INTRODUCTION

Previously, the isolation and characterization of about two dozen polyoxygenated xanthones [1] and two tetracyclic triterpenes [2] from the different parts of *Canscora decussata* (Gentianaceae) were reported. Subsequently, medicinal importance of some of these xanthones were also reported [3–5]. The present paper describes the isolation and structure elucidation of one new xanthone and its glycoside occurring in the leaves and stems of this species.

RESULTS AND DISCUSSION

Extraction of the defatted plant material with EtOH followed by partitioning of the alcoholic extractives in water and solvents of graded polarity yielded 5 xanthone fractions (Fractions A–E). Column chromatography of the individual fractions over Si gel or florisil afforded a number of new and known [1] xanthones and xanthone glycosides. The characterization of a new xanthone and its *O*-glycoside is described here.

Xanthone-1. This compound, mp 354–355°, $C_{14}H_{10}O_5$ (M^+, 258), was a monomethoxydihydroxyxanthone in which the two hydroxyl groups were not chelated (PMR spectrum). It formed a dimethyl ether with ethereal diazomethane and a diacetate. The UV spectrum was characteristic of 1,3,5-trioxygenated xanthones [6]. The compound was soluble in dilute Na_2CO_3 solution and showed a strong bathochromic shift of the K-band on addition of NaOAc, typical of 3-hydroxyxanthones [7]. The methoxyl group was placed at the C-1 position since no UV spectral shift was observed upon addition of $AlCl_3$. The 60 MHz PMR spectrum in DMSO-d_6 showed two *meta* split protons at δ 6.55 and 6.40, their position and coupling suggested 1,3-dioxygenation [8] in the A-ring of the xanthone. The lowest field proton, centered at δ 7.5, appeared as a quartet with splittings of about 6 and 4 Hz, while the two remaining protons appeared around δ 7.2 as a complex multiplet suggesting an ABC system associated with C-8, C-7 and C-6 protons. The MS of the xanthone was also consistent with a C-1 methoxy group. Aside from the molecular ion peak, significant fragment ion peaks appeared from the loss of OH, H_2O, and CHO from the molecular ion. Similar observations were made [9] for xanthones and related aromatic compounds with a methoxy substituent *peri* to a carbonyl group. Xanthone-I was therefore assigned the structure 1-methoxy-3,5-dihydroxyxanthone (**1**). For confirmation, 1-hydroxy-3,5-dibenzyloxyxanthone was methylated with dimethyl sulphate and alkali, debenzylation of the product by catalytic hydrogenation gave 1-methoxy-3,5-dihydroxyxanthone identical with the natural product in all respects. C-1 methoxylated xanthones are comparatively rare in nature.

Xanthone-2. This compound, mp 252–255°, $C_{26}H_{30}O_{14}.H_2O$, was a monohydroxy-monomethoxy-xanthone-*O*-disaccharide as evidenced from its PMR spectrum and formation of an octamethyl ether and a heptaacetate. It showed UV absorption characteristic of 1,3,5-trioxygenated xanthones. On acid hydrolysis it furnished 1-methoxy-3,5-dihydroxyxanthone, glucose, and rhamnose. In the PMR spectrum of the heptaacetate, one acetoxyl group appeared at a considerably lower field than the other six suggesting its assignment as an aromatic acetoxyl. The signals from the aromatic protons at C-8, C-7 and C-6, in the acetate derivative were the same as those for the corresponding protons of 1-methoxy-3,5-diacetoxyxanthone, while those from the C-2 and C-4 positions differed significantly from the latter compound. On this basis it appeared that the glycoside linkage was present in ring-A and therefore at C-3. This assignment is also consistent with the observations that xanthone-II did not respond to $FeCl_3$ test and there were no shifts in the UV absorption maxima in the presence of either NaOAc or $AlCl_3$. To obtain further confirmation, the compound was permethylated followed by acid hydrolysis when the aglycone was identified as

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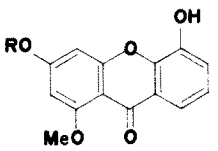
1,5-dimethoxy-3-hydroxyxanthone. The structure of the glycosyl moiety was established by PMR spectral analysis [10] of the compound and its heptaacetate which indicated that the compound was 1-methoxy-5-hydroxyxanthone-3-*O*-rutinoside (**2**). The two compounds (**1** and **2**) have not been encountered before in nature or prepared synthetically. Also, this is the first demonstration of the occurrence of a xanthone-*O*-rutinoside in nature and a xanthone-disaccharide in the genus *Canscora*.



(**1**) R = H

(**2**) R = rutinosyl

EXPERIMENTAL

The general methods are the same as reported in a recent paper [11].

Extraction of C. decussata. Dried and milled leaves and stems of *Canscora decussata* Schult* (2 kg) were continuously extracted in a Soxhlet with petrol (60–80°) and then with EtOH (30 hr each). The isolation of xanthones from the petrol extractives was previously reported [1,2].

Treatment of the EtOH extract. The EtOH extract was conc under red. press. when mangiferin was separated as a light yellow solid [3]. The EtOH mother liquor was further conc to a syrupy liquid and 4% HOAc (400 ml) added. The mixture was kept at room temp. overnight. The aq. acidic suspension was extracted with Et_2O (5 × 250 ml) and the combined Et_2O extracts evaporated to give a dull yellow residue, consisting of a mixture of xanthones (Fraction A, 4.3 g). The aq. layer was conc (*ca.* 100 ml) and extracted with EtOAc (5 × 250 ml). The residue from the combined EtOAc extracts, a light brown solid (Fraction B, 0.21 g), contained one major and two minor xanthones. The aq. mother liquor was then basified (NH_4OH) and again extracted with Et_2O, EtOAc, and *n*-BuOH (2 × 250 ml each). The first two extractives (Fractions C and D) were not processed further at this stage. The combined BuOH extracts were evaporated to give a dark brown solid (Fraction E, 0.3 g).

Xanthone-I (**1**). A portion of the residue (0.1 g) from Fraction B was crystallized from MeOH as light brown needles (68 mg), mp 354–355°; R_f 0.77 (*n*-BuOH–HOAc–H_2O, 4:1:2); UV λ_{max}^{EtOH} nm (o.d.): 245 (0.59), 288 (0.18), 305 (0.16), 336 (0.27); IR ν_{max}^{KBr} cm^{-1}: 1655, 1610, 1588; PMR (DMSO-d_6): δ 3.88 (3H, *s*, C-1 OMe), 6.40 (1H, *d*, *J* 3 Hz, C-2), 6.55 (1H, *d*, *J* 3 Hz, C-4), 7.20 (2H, *m*, C-7, C-6), 7.52 (1H, *q*, *J* 6, 4 Hz, C-8); MS *m/e* (rel. int.): 258 (M^+, 100), 241 (22, 258 → 241 transition, m* 225.5), 240 (8), 229 (84), 228 (53), 200 (21), 171 (9), 155 (4), 115 (18). (Found: C, 64.88; H, 3.54. $C_{14}H_{10}O_5$ requires: C, 65.11; H, 3.5%).

Acetylation of xanthone-I with Ac_2O–C_5H_5N yielded the diacetate from CH_2Cl_2/hexane, mp 145–146°; PMR ($CDCl_3$): δ 2.35 (3H, *s*, C-5 OAc), 2.45 (3H, *s*, C-3 OAc), 4.0 (3H, *s*, C-1 OMe), 6.65 (1H, *d*, *J* 2.5 Hz, C-2), 6.90 (1H, *d*, *J* 2.5 Hz C-4), 7.40 (2H, *m*, C-7, C-6), 8.2 (1H, *q*, C-8). The permethyl ether, prepared with ethereal CH_2N_2, crystallized from EtOH as colourless needles, mp 220–221°; UV λ_{max}^{EtOH} nm (o.d.): 245 (0.45), 300 (0.21), 335 (0.12); IR γ_{max}^{Nujol} cm^{-1}: 1650, 1620, 1608, 1598. Mixed mp with 1,3,5-trimethoxyxanthone, mp 219–220°, and comparison by UV, IR, and TLC confirmed the identity.

1-*Hydroxy*-3,5-*dibenzyloxyxanthone.* 1,3,5-Trihydroxyxanthone (0.4 g) [3], K_2CO_3 (1.5 g) and benzyl chloride (1 ml) in dry Me_2CO (20 ml) were refluxed for 4 hr. The reaction was cooled, diluted with H_2O, and extracted with $CHCl_3$ (3 × 50 ml). The $CHCl_3$ extract was worked up, conc, and filtered through Si gel (BDH, 60–120 mesh, 1.1 × 20 cm) when 1-hydroxy-3,5-dibenzyloxyxanthone was obtained as orange micro needles (42 mg), mp 135–137° (M^+, 424).

1-*Methoxy*-3,5-*dihydroxyxanthone.* 1-Hydroxy-3,5-dibenzyloxyxanthone (38 mg) was methylated with Me_2SO_4 (1 ml) and K_2CO_3 (2 g) in dry AcMe (50 ml) under reflux (40 hr) to give 1-methoxy-3,5-dibenzyloxyxanthone as a brown amorphous solid (32 mg). It was dissolved in EtOH (50 ml) and subjected to catalytic hydrogenation in the presence of PtO_2 (50 mg). The product was worked up in the usual way and crystallized from MeOH to give 1-methoxy-3,5-dihydroxyxanthone as brown needles (7 mg), mp 355–357°; mixed mp with xanthone-I 354–355°; comparison by IR and TLC confirmed the identity.

Separation of xanthones from Fraction E. A portion of the solid (0.1 g) was dissolved in MeOH and chromatographed over florisil (1.1 × 18 cm) using Et_2O, MeOH and different mixtures of these solvents as eluents. The Et_2O–MeOH (2:1) eluates on evaporation gave xanthone-II admixed with a minor component which was removed by crystallization.

Xanthone-II (**2**). Crystallized from MeOH–AcMe as straw coloured needles, mp 252–255 (44 mg); R_f 0.32; $[\alpha]_D^{22}$ −88° (*c* 0.42, pyridine); UV: λ_{max}^{EtOH} nm (o.d.): 244 (0.71), 270 *sh* (0.65), ~325 (0.35); PMR (DMSO-d_6): δ 0.85 (3H, *m*, rhamnosyl Me), 3.6 (10H, rhamnoglucosyl), 3.88 (3H, *s*, C-1 OMe), 4.22 (1H, *d*, *J* 2 Hz, rhamnosyl C-1), 5.0 (1H, *br*, glucosyl C-1), 6.38 (1H, *d*, *J* 3 Hz, C-2), 6.54 (1H, *d*, *J* 3 Hz, C-4), 7.22 (2H, *m*, C-7, C-6), 7.58 (1H, *q*, C-8) (Found: C, 53.02; H, 4.98. $C_{26}H_{30}O_{14}.H_2O$ requires: C, 53.42; H, 5.13%).

Xanthone-II (32 mg) was hydrolyzed with H_2SO_4 (3%, 10 ml) for 30 min on a steam bath. The reaction on cooling furnished 1-methoxy-3,5-dihydroxyxanthone as a brown ppt. which was collected by filtration. The filtrate was neutralized and conc. TLC and PPC [11] of the aq. concentrate showed the presence of glucose and rhamnose. The sugars were detected with metaperiodate-benzidine reagent.

Xanthone-II was acetylated with Ac_2O–C_5H_5N (1:1) for 1 hr on a steam bath. Work up and crystallization from CH_2Cl_2–hexane gave the heptaacetate, mp 200–202°; PMR ($CDCl_3$): δ 1.15 (3H, rhamnosyl Me), 1.98–208 (18H, rhamnoglucosyl acetoxyl), 2.44 (3H, Ar-acetoxyl), 3.90 (1H, rhamnosyl), 3.95 (5H, C-1-OMe, glucosyl), 4.80 (1H, rhamnosyl C-1), 5.32 (7H, *m*, rhamnosyl and glucosyl), 6.75 (2H, C-2, C-4), 7.42 (2H, *m*, C-7, C-6), 8.23 (1H, *m*, C-8).

A portion of xanthone-II (48 mg) was methylated with MeI and NaH in THF at room temp according to the method of Stoochnoff and Benoiton [12]. The product showed one major and two minor spots on TLC ($CHCl_3$–HOAc, 99:1). The major component was separated by chromatography over deactivated Si gel G (E. Merck). It was obtained as a yellow amorphous solid on evaporation of C_6H_6–$CHCl_3$ eluates. The permethyl ether was hydrolyzed (3% H_2SO_4) when 1,5-dimethoxy-3-hydroxy-xanthone was obtained as a yellow solid. It crystallized from EtOH as yellow needles, mp 227–228° (18 mg); UV λ_{max}^{EtOH} nm (o.d.): 248 (0.42), 315 (0.14), 355 (0.15); $\lambda_{max}^{EtOH-NaOAc}$ nm: 250, 275, 378; MS *m/e* (rel. int.): 272 (M^+, 100), 257 (12), 255 (16), 254 (6), 243 (42), 229 (22). On methylation with ethereal CH_2N_2 it gave 1,3,5-trimethoxyxanthone, mp and mixed mp 219–220°; co-TLC.

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