New Antifungal Xanthones from the Seeds of *Rhus coriaria* L.

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Z. Naturforsch. **66c**, 17–23 (2011); received June 18/September 6, 2010

Phytochemical investigations of the ethanolic extract of the seeds of *Rhus coriaria* L. (Anacardiaceae) led to the identification of four new xanthones, characterized as 2,3-dihydroxy-7-methyl xanthone (**1**), 2,3,6-trihydroxy-7-hydroxymethylene xanthone-1-carboxylic acid (**2**), 2-methoxy-4-hydroxy-7-methyl-3-*O*-*β*-D-glucopyranosyl xanthone-1,8-dicarboxylic acid (**4**), and 2-hydroxy-7-hydroxymethylene xanthone-1,8-dicarboxylic acid 3-*O*-*β*-D-glucopyranosyl-(2'→3'')-3''-*O*-stigmast-5-ene (**5**), along with the known steroidal glucoside *β*-sitosterol-*β*-D-glucoside (**3**). The structures of the isolated compounds have been identified on the basis of spectral data analysis and chemical reactions. All xanthones were active against *Aspergillus flavus*.

Key words: Rhus coriaria L., Xanthones, Antifungal Activity

Introduction

Rhus coriaria L. (Anacardiaceae), commonly known as sumac, is a deciduous shrub growing up to 3 m in height in Mediterranean regions, North Africa, Southern Europe, Iran, and Afghanistan (Kurucu *et al*., 1993). Sumac leaves are used as a condiment and for tanning leather; the fruits are prescribed to relieve stomach diseases, bowl complaints, fever, dermatitis, and as an appetizer, diuretic, and antiseptic (Altinkurt and Heper, 1970; Rayne and Mazza, 2007; Ozcan and Haciseferogullari, 2004). Sumac is beneficial to prevent diabetes, hyperglycaemia, obesity, paralysis, colitis, and diarrhoea (Giancario *et al*., 2006; Kirtikar and Basu, 2000). The seeds are appetizer, astringent, diuretic, styptic, and tonic, and are prescribed to treat dysentery, haemoptysis, and conjunctivitis (Chopra *et al*., 1986). Fatty acids, flavonoids, and volatile components have been reported from sumac seeds and fruits (Brunke *et al*., 1993; Rayne, 2008; Mehrdad *et al*., 2009; Mavlyanov *et al*., 1997; Bahar and Altug, 2009; Dogan and Akgul, 2006). The present paper describes the isolation and characterization of four new xanthones and one known compound from the ethanolic extract of seeds of this plant and screening of their antifungal activity.

Material and Methods

General

Melting points were determined on a Perfit melting point apparatus (Ambala, India) and are uncorrected. IR spectra were recorded using KBr discs, with a Bio-Rad FT-IR 5000 spectrometer (FTS 135, Hongkong). UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. ^{1}H and ^{13}C NMR spectra were registered using Bruker Advance DRY 400 spectrospin and Bruker Advance DRY 100 spectrospin instruments (Rheinstetten, Germany), respectively, in DMSO-d_6 and with TMS as an internal standard. FAB mass spectra were obtained using a JEOL-JMS-DX 303 spectrometer (Peabody, MA, USA). Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh. TLC was run on silica gel G (Qualigens). Spots were visualized by exposure to iodine vapour, UV radiation, and by spraying reagents.

Plant material

The seeds of *R. coriaria* were purchased from Khari Baoli, a local market of Delhi, India, and authenticated by Dr. M. P. Sharma, Department of Botany, Jamia Hamdard, New Delhi, India. A voucher specimen (No. PRL/JH/03/22) is deposit-

0939–5075/2011/0100–0017 $ 06.00

ed in the herbarium section of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India.

Extraction and isolation

Air-dried seeds (2 kg) were coarsely powdered, defatted with petroleum ether, and then exhaustively extracted with ethanol (95%). The combined extracts were concentrated on a water bath and dried under reduced pressure to get 110 g (5.5% yield) of a dark brown mass. The viscous dark brown mass was dissolved in a small quantity of methanol and adsorbed on silica gel (60–120 mesh) for preparation of a slurry. It was dried, packed, and chromatographed over a silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, chloroform, and methanol, *i.e.* with solvents of increasing polarity to isolate the following compounds:

2,3-Dihydroxy-7-methyl xanthone (**1**)

Elution from the column with $CHCl_3$/MeOH (9:1) afforded a light yellow amorphous powder which was recrystallized from MeOH; yield: 470 mg (0.0235%). – R_f = 0.75 ($CHCl_3$/acetone/MeOH, 7:2:1). – M.p. 270–272 °C. – UV (MeOH): λ_{max} = 234, 254, 278, 330 nm (log ε = 4.8, 5.5, 3.2, 1.3). – IR (KBr): ν_{max} = 3448, 2950, 2861, 1657, 1541, 1520, 1218, 930 cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 7.97 (1H, d, J = 8.4 Hz, H-5), 7.87 (1H, brs, H-4), 7.01 (1H, d, J = 3.0 Hz, H-8), 6.93 (1H, dd, J = 8.4, 3.0 Hz, H-6), 6.79 (1H, brs, H-1), 6.75 (1H, brs, D_2O exchangeable, OH), 6.55 (1H, brs, D_2O exchangeable, OH), 2.50 (3H, brs, CH_3-10). – ^{13}C NMR (DMSO-d_6): δ = 129.55 (C-1), 164.05 (C-2), 161.42 (C-3), 93.95 (C-4), 159.27 (C-4a), 148.61 (C-4b), 115.96 (C-5), 127.14 (C-6), 130.97 (C-7), 102.56 (C-8), 107.52 (C-8a), 181.64 (C-9),102.56 (C-9a), 28.99 (C-10). – FAB MS: m/z = 242 $[M]^+$ ($C_{14}H_{10}O_4$).

2,3,6-Trihydroxy-7-hydroxymethylene xanthone-1-carboxylic acid (**2**)

Elution from the column with $CHCl_3$/MeOH (17:3) afforded a light brown amorphous powder which was recrystallized from acetone; yield: 580 mg (0.029%). – R_f = 0.74 ($CHCl_3$/MeOH, 7:3). M.p. 295 °C (dec.). – UV (MeOH): λ_{max} = 202, 231, 255, 279, 335 nm (log ε = 3.8, 4.6, 5.3, 3.1, 1.1). – IR (KBr): ν_{max} = 3410, 3360, 3280, 2950, 2860, 1690, 1663, 1560, 1219, 930 cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 13.24 (1H, brs, COOH), 8.15 (1H, brs, H-4), 7.56 (1H, brs, H-5), 7.08 (1H, brs, H-8), 3.43 (2H, brs, H_2-11). – ^{13}C NMR (DMSO-d_6): δ = 130.52 (C-1), 166.15 (C-2), 159.83 (C-3), 92.06 (C-4), 157.19 (C-4a), 152.66 (C-4b), 115.38 (C-5), 151.63 (C-6), 137.85 (C-7), 103.58 (C-8), 109.08 (C-8a), 180.52 (C-9), 101.83 (C-9a), 183.04 (C-10), 65.22 (C-11). – FAB MS: m/z = 318 $[M]^+$ ($C_{15}H_{10}O_8$).

β-Sitosterol-β-D-glucoside (**3**)

Elution from the column with $CHCl_3$/MeOH (93:7) furnished a colourless amorphous powder which was recrystallized from MeOH; yield: 245 mg (0.0122%). – M.p. 270–272 °C. – R_f = 0.53 (C_6H_6/$CHCl_3$/MeOH, 5:4:1). – UV (MeOH): λ_{max} = 268 nm (log ε = 4.5). – IR (KBr): ν_{max} = 3450, 2955, 1610, 1460, 1375, 1255, 1155, 1100, 1080, 1020 cm^{-1}. – FAB MS: m/z (rel. int.) = 576 $[M]^+$ ($C_{35}H_{60}O_6$) (1.5), 413 $[M - sugar]^+$ ($C_{29}H_{50}O$) (4.3).

2-Methoxy-4-hydroxy-7-methyl-3-*O*-β-D-glucopyranosyl xanthone-1,8-dicarboxylic acid (**4**)

Elution from the column with $CHCl_3$/MeOH (4:1) afforded a light brown amorphous powder which was recrystallized from methanol; yield: 240 mg (0.012%). – R_f = 0.70 ($CHCl_3$/acetone/MeOH, 6:2:2). – M.p. 251–253 °C. – UV (MeOH): λ_{max} = 202, 223, 255, 281, 335 nm (log ε = 5.1, 4.9, 3.1, 1.3, 1.5). – IR (KBr): ν_{max} = 3447, 3350, 3290, 2850, 1701, 1689, 1670, 1541, 1527, 1470, 1218, 930 cm^{-1}. – ^{1}H NMR (DMSO-d_6): δ = 7.20 (1H, d, J = 8.1 Hz, H-5), 7.04 (1H, d, J = 8.1 Hz, H-6), 5.02 (1H, d, J = 6.9 Hz, H-1'), 4.86 (1H, m, H-5'), 4.65 (1H, dd, J = 6.9, 5.4 Hz, H-2'), 3.65 (1H, m, H-3'), 3.47 (3H, brs, OCH_3), 3.13 (1H, m, H-4'), 3.03 (1H, d, J = 7.5 Hz, H_2-6'a), 3.01 (1H, d, J = 7.5 Hz, H_2-6'b), 2.13 (3H, brs, H_3-11). – ^{13}C NMR (DMSO-d_6) δ = 131.15 (C-1), 158.37 (C-2), 156.35 (C-3), 149.38 (C-4), 159.16 (C-4a), 147.51 (C-4b), 117.40 (C-5), 128.96 (C-6), 131.15 (C-7), 130.17 (C-8), 110.15 (C-8a), 182.57 (C-9), 110.15 (C-9a), 179.75 (C-10), 27.73 (C-11), 181.07 (C-12), 100.55 (C-1'), 75.97 (C-2'), 73.34 (C-3'), 69.84 (C-4'), 76.53 (C-5'), 60.95 (C-6'), 56.44 (OMe). – +ve FAB MS: m/z (rel. int.) = 522 $[M]^+$ ($C_{23}H_{22}O_{14}$) (10.5), 477 (9.2), 388 (16.3), 359 (75.6), 344 (100), 181 (63.2), 136 (21.3).

2-Hydroxy-7-hydroxymethylene xanthone-1,8-dicarboxylic acid 3-*O*-β-D-glucopyranosyl-(2'→3'')-3''-*O*-stigmast-5-ene (**5**)

Elution from the column with $CHCl_3$/MeOH (3:1) furnished a light brown coloured mass which was recrystallized from acetone/MeOH (9:1);

yield: 610 mg (0.0305%). – R_f = 0.85 (toluene/ethyl acetate/formic acid, 5:4:1). – M.p. 280–281 °C. – UV (MeOH): λ_{max} = 210, 235, 280, 335 nm (log ε = 5.7, 3.3, 1.3, 1.4). – IR (KBr): ν_{max} = 3410, 3380, 3250, 2950, 2355, 1705, 1690, 1665, 1541, 1350, 1260, 930 cm^{-1}. – ^1H NMR (DMSO-d_6): δ = 13.29 (1H, brs, COOH), 13.06 (1H, brs, COOH), 7.84 (1H, brs, H-4), 7.11 (1H, d, J = 8.5 Hz, H-5), 6.92 (1H, d, J = 8.5 Hz, H-6), 5.38 (1H, m, H-6''), 4.96 (1H, d, J = 7.1 Hz, H-1'), 4.51 (1H, brm, H-5'), 4.25 (1H, brm, $w_{1/2}$ = 16.8 Hz, H-3''b), 4.19 (1H, m, H-2'), 3.72 (1H, m, H-3'), 3.62 (1H, m, H-4'), 3.47 (2H, brs, H_2-11), 3.19 (2H, brs, H_2-6'), 1.08 (3H, brs, Me-19''), 0.96 (3H, d, J = 6.5 Hz, Me-21''), 0.80 (3H, d, J = 6.0 Hz, Me-29''), 0.86 (6H, brs, Me-26'', Me-27''), 0.70 (3H, brs, Me-18''). – ^{13}C NMR (DMSO-d_6): δ = 137.81 (C-1), 165.28 (C-2), 161.87 (C-3), 92.89 (C-4), 159.23 (C-4a), 158.76 (C-4b), 114.42 (C-5), 126.58 (C-6), 130.89 (C-7), 143.59 (C-8), 107.26 (C-8a), 179.81 (C-9), 109.25 (C-9a), 183.11 (C-10), 65.81 (C-11), 181.19 (C-12), 101.23 (C-1'), 75.05 (C-2'), 71.25 (C-3'), 69.52 (C-4'), 75.84 (C-5'), 60.17 (C-6'), 37.17 (C-1''), 21.80 (C-2''), 71.78 (C-3''), 41.40 (C-4''), 141.23 (C-5''), 125.11 (C-6''), 33.93 (C-7''), 40.25 (C-8''), 48.05 (C-9''), 36.90 (C-10''), 21.03 (C-11''), 38.28 (C-12''), 38.55 (C-13''), 54.63 (C-14''), 23.88 (C-15''), 29.84 (C-16''), 53.85 (C-17''), 10.14 (C-18''), 19.02 (C-19''), 35.24 (C-20''), 18.04 (C-21''), 34.64 (C-22''), 26.19 (C-23''), 43.63 (C-24''), 27.65 (C-25''), 22.28 (C-26''), 17.25 (C-27''), 16.94 (C-28''), 10.16 (C-29''). – FAB MS: *m/z* (rel. int.) = 925 $[M+H]^+$ ($C_{51}H_{89}O_{14}$) (5.8), 413 (24.3), 512 (26.9).

Measurement of antifungal activity

The antifungal activity was determined on *Aspergillus flavus* (MTCC-277), *Candida albicans* (MTCC-3958), and *Penicillium citrinum* (MTCC-3395). A fungal suspension in sterile normal saline was prepared. An aliquot of 1.5 ml was uniformly seeded on the malt extract media (15 ml, 4 cm thick) in Petri dishes, left aside for 15 min, and excess was drained and discarded properly (Kar *et al*., 1999). Wells of 6 mm diameter and about 2 cm apart were punctured into culture media using a sterile cork borer (6 mm). Concentrations of 25, 50, 100, and 200 μg/ml of test compounds were prepared in dimethyl sulfoxide (DMSO). The standard drug fluconazole (32-mg tablet) was obtained from Cipla Laboratories (Mumbai, India). The plates were then incubated at 30 °C for 48 h. After incubation, bioactivity was determined by measuring the diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Controls included solvent without test compounds, although no antifungal activity was noted in the solvent employed for the test.

Results and Discussion

Compound **1** was obtained as a light yellow amorphous powder from chloroform/methanol (9:1). The FAB mass analysis of **1** indicated a molecular formula of $C_{14}H_{10}O_4$. Its UV spectrum showed absorption maxima at 234, 254, 278, and 330 nm in methanol suggesting **1** to be a xanthone. The UV absorption bands did not show any bathochromic shift on addition of $AlCl_3$ solution, indicating the absence of hydroxy groups at C-1 and C-8. A bathochromic shift of the band at 278 nm to 295 nm with sodium acetate solution supported the presence of a hydroxy group at C-3 (Odontuya *et al*., 1998; Ghosal *et al*., 1975). Its IR spectrum exhibited characteristic absorption bands for a hydroxy group (3448 cm^{-1}), carbonyl group (1657 cm^{-1}), and an aromatic ring (1541, 1520, and 930 cm^{-1}). The ^1H NMR spectrum of **1** exhibited two one-proton broad signals at δ 6.79 and 7.87 ppm assigned to *p*-coupled H-1 and H-4, respectively, two one-proton doublets at δ 7.97 (J = 8.4 Hz) and 7.01 ppm (J = 3.0 Hz) ascribed to H-5 and H-8, respectively, an one-proton double doublet at δ 6.93 ppm (J = 8.4, 3.0 Hz) attributed to H-6, and a three-proton broad signal at δ 2.50 ppm accounted to C-10 methyl protons located on the aromatic nucleus. The ^{13}C NMR spectral data were in accordance with those of xanthone molecules (Dall'Acqua *et al*., 2004; Pinheiro *et al*., 1998; Purev *et al*., 2002). Protonated carbon atoms were assigned by an HMQC spectrum where crossed peaks were observed between H-8 and C-4b and C-9. Further long-range correlations were observed between the protons H_3-10, H-6 and H-8 and the carbon atom C-7 indicating the location of the methyl function at C-7. Diagnostic long-range correlations were observed between H-8 and C-4b and C-9. Further long-range correlations were observed between H-5 and C-4b and C-8b; H-1 and C-9, C-9a, and C-2. Hence, the structure of **1** has been established as 2,3-dihydroxy-7-methyl xanthone (Fig. 1), named coriarianxanthonediol. It is a new natural compound.

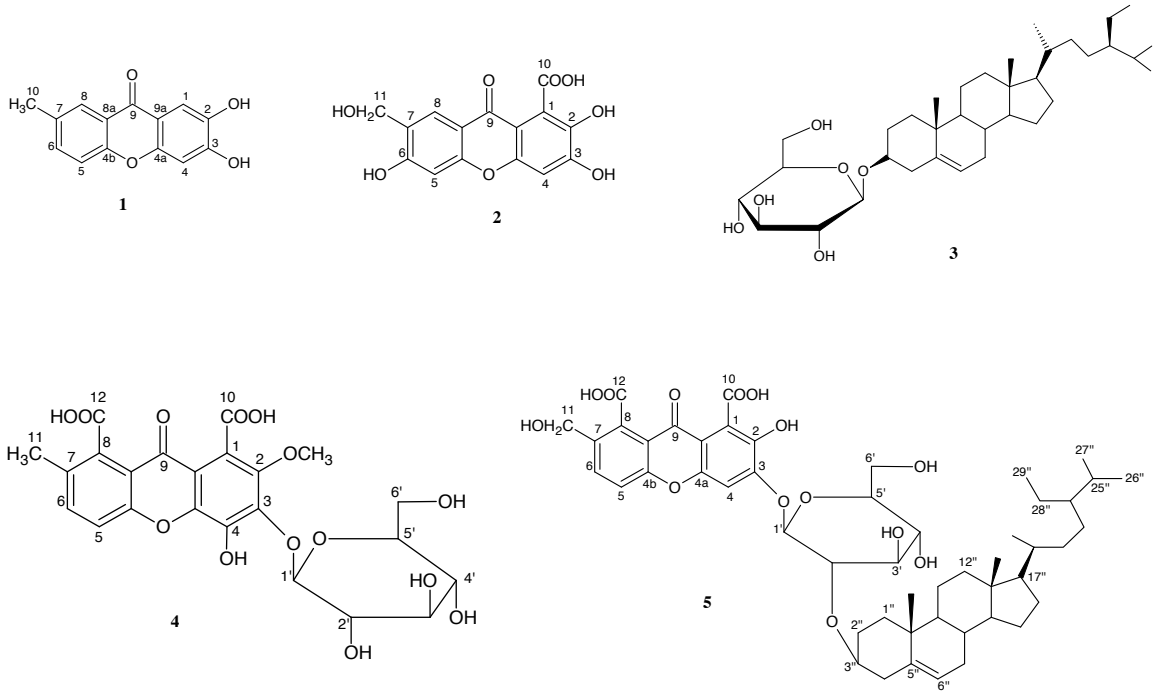

Fig. 1. Chemical structures of the isolated compounds 2,3-dihydroxy-7-methyl xanthone (**1**), 2,3,6-trihydroxy-7-hydroxymethylene xanthone-1-carboxylic acid (**2**), *β*-sitosterol-*β*-D-glucoside (**3**), 2-methoxy-4-hydroxy-7-methyl-3-*O*-*β*-D-glucopyranosyl xanthone-1,8-dicarboxylic acid (**4**), and 2-hydroxy-7-hydroxymethylene xanthone-1,8-dicarboxylic acid 3-*O*-*β*-D-glucopyranosyl-(2'→3'')-3''-*O*-stigmast-5-ene (**5**).

Compound **2** was obtained as a light brown amorphous powder from chloroform/methanol (17:3). FAB MS indicated the molecular formula $C_{15}H_{10}O_8$ with a molecular ion peak at *m/z* 318. The UV absorption maxima at 202, 231, 255, 279, and 335 nm suggested that **2** was a xanthone. Neither bathochromic nor hypsochromic shifts were observed in the UV spectrum with $AlCl_3$ indicating the absence of hydroxy groups at C-1 and C-8. The UV spectrum showed a bathochromic shift of the absorption maximum at 335 to 360 with ethanolic sodium acetate, indicating the presence of 3-OH (Markham, 1982; Purev *et al.*, 2002). The IR spectrum of **2** showed characteristic absorption bands for a carboxylic group (3280, 1690 cm^{-1}), hydroxy groups (3410, 3360 cm^{-1}), and a carbonyl group (1663 cm^{-1}). The ^1H NMR spectrum of **2** exhibited three one-proton broad signals at δ 8.15, 7.56, and 7.08 ppm assigned to aromatic H-4, H-5, and H-8 protons and a two-proton broad signal at δ 3.43 ppm ascribed to hydroxymethylene H_2-11 protons. The ^{13}C NMR spectrum of **2** exhibited 15 carbon signals for 12 aromatic carbon atoms, and one of each for carbonyl (δ 180.52 ppm), carboxylic (δ 183.04 ppm), and hydroxymethylene (δ 65.22 ppm) carbon atoms. The protonated carbon atoms were assigned by an HMQC spectrum while its HMBC spectrum exhibited correlation of C-9 with H-8; C-7 with H-5, H-8 and H-11; C-4a with H-4; and C-4b with H-5. From the above spectral evidences, compound **2** was identified as 2,3,6-trihydroxy-7-hydroxymethylene xanthone-1-carboxylic acid (Fig. 1), named coriariaxanthonoic acid. It is a new phytoconstituent.

Compound **4** was isolated from chloroform/methanol (4:1) as a light brown amorphous powder. A molecular ion peak at *m/z* 522 was analysed by FAB MS according to the molecular formula $C_{23}H_{22}O_{14}$. It turned red in the presence of $MgCl_2$ which is characteristic for a xanthone glucoside (Markham, 1982; Purev *et al.*, 2002). There was no bathochromic or hypsochromic shift in the presence of $AlCl_3$ indicating the absence of hydroxy groups at positions C-1 and C-8. The UV spectrum did not show a bathochromic shift of the longest wavelength maximum with

ethanolic sodium acetate indicating the location of a glycosidic unit at C-3 (Purev *et al.*, 2002). Its IR spectrum showed characteristic absorption bands for hydroxy groups (3447, 3350 cm^{-1}), carboxylic groups (3290, 1701, 1689 cm^{-1}), and a keto group (1670 cm^{-1}). It gave effervescences with sodium bicarbonate solution supporting the presence of a carboxylic group in the molecule. The ^{1}H NMR spectrum displayed two one-proton doublets at δ 7.20 (J = 8.1 Hz) and 7.04 ppm (J = 8.1 Hz) assigned to *ortho*-coupled H-5 and H-6 protons, respectively, a three-proton broad signal at δ 3.47 ppm ascribed to methoxy protons, sugar protons from δ 5.02 to 3.01 ppm and a three-proton broad signal at δ 3.47 ppm ascribed to methoxy protons, and a three-proton broad signal at δ 2.13 ppm attributed C-11 methyl protons. The ^{13}C NMR spectrum exhibited signals for carboxylic carbon atoms at δ 179.75 (C-10) and 181.07 ppm (C-12), a carbonyl carbon atom at δ 182.57 ppm (C-9), aromatic carbon atoms from δ 159.16 to 110.15 ppm, an anomeric carbon atom at δ 100.55 ppm (C-1'), other sugar carbon atoms from δ 76.53 to 60.95 ppm, a methoxy carbon atom at δ 56.44 ppm, and a methyl carbon atom at δ 27.73 ppm. The HMBC spectrum of **4** showed correlations of C-7 with H-5, H-6 and H_3-11; and C-3 with H-1'. After acidic hydrolysis with 5% HCl, the sugar residue was determined by paper chromatography as D-glucose. From the above spectral and chemical evidences, the structure of **4** has been characterized as 2-methoxy-4-hydroxy-7-methyl-3-*O*-β-D-glucopyranosyl xanthone-1,8-dicarboxylic acid (Fig. 1). This is a new xanthone glucoside.

Compound **5** was obtained as a light brown mass from chloroform/methanol (3:1). Its molecular mass was determined to be 925 by FAB MS consistent with the molecular formula of xanthonyl glucosidosterol, $C_{51}H_{89}O_{14}$. Its UV spectral data did not show any shift with $AlCl_3$ and ethanolic sodium acetate suggesting the presence of a glycosidic unit at C-3. It produced effervescences with sodium bicarbonate solution and had characteristic IR absorption bands for hydroxy groups (3410, 3380 cm^{-1}), carboxylic groups (3250, 1705, and 1690 cm^{-1}), and a carbonyl group (1665 cm^{-1}). The ^{1}H NMR spectrum of **5** showed an one-proton broad signal at δ 7.84 ppm assigned to H-4, two one-proton doublets at δ 7.11 (J = 8.5 Hz) and 6.92 ppm (J = 8.5 Hz) ascribed to *ortho*-coupled H-5 and H-6, respectively, an one-proton multiplet at δ 5.38 ppm attributed to vinylic H-6'', an one-proton doublet at δ 4.96 ppm (J = 7.1 Hz) accounted to anomeric H-1', and other oxygenated methine and methylene protons from δ 4.51 to 3.19 ppm. Two three-proton signals at δ 1.08 and 0.70 ppm and a six-proton broad signal at δ 0.86 ppm were associated with the tertiary C-19'' and C-18'', and with the secondary C-26'' and C-27'' methyl protons. Two three-proton doublets at δ 0.96 (J = 6.5 Hz) and 0.80 ppm (J = 6.0 Hz) were due to secondary C-21'' and primary C-29'' methyl protons. The ^{13}C NMR spectrum of **5** exhibited signals for a carbonyl carbon atom at δ 179.81 ppm (C-9), carboxylic carbon atoms at δ 183.11 (C-10) and 181.19 ppm (C-12), aromatic and vinylic carbon atoms from δ 165.28 to 92.89 ppm, anomeric carbon atom at δ 101.23 ppm (C-1'), and other sugar carbon atoms between δ 75.84 and 60.17 ppm. The carbon signals at δ 71.78 ppm (C-3'') and between δ 54.63 and 10.16 ppm were due to β-sitosterol which was compared with the reported values (Ali, 2001; Greca *et al.*, 1990). Protonated carbon signals were assigned by the HMQC spectrum. The appearance of the sugar C-2' proton in the deshielded region at δ 4.19 ppm in the ^{1}H NMR spectrum and at δ 75.05 ppm in the ^{13}C NMR

Table I. Antifungal activity of isolated compounds **1**, **2**, **4**, and **5**.

Compound	Concentration [μg/ml]	Mean zone of inhibition [mm][a]		
		Aspergillus flavus	*Candida albicans*	*Penicillium citrinum*
1	25	NIL	NIL	NIL
	50	12 ± 0.1	NIL	NIL
	100	13 ± 0.2	NIL	NIL
	200	13 ± 0.2	NIL	NIL
2	25	10 ± 0.1	12 ± 0.1	NIL
	50	10 ± 0.2	12 ± 0.1	NIL
	100	11 ± 0.1	12 ± 0.1	NIL
	200	11 ± 0.1	13 ± 0.2	NIL
4	25	12 ± 0.1	11 ± 0.1	10 ± 0.1
	50	12 ± 0.1	12 ± 0.1	11 ± 0.1
	100	12 ± 0.2	18 ± 0.2	11 ± 0.1
	200	13 ± 0.1	19 ± 0.2	12 ± 0.2
5	25	11 ± 0.1	13 ± 0.1	10 ± 0.1
	50	12 ± 0.1	14 ± 0.2	10 ± 0.1
	100	12 ± 0.2	15 ± 0.2	11 ± 0.1
	200	13 ± 0.2	17 ± 0.2	13 ± 0.1
Fluconazole	32	19 ± 0.2	18 ± 0.1	18 ± 0.1

NIL, No antifungal activity.
[a] Values are averages of three replicates ± SEM.

spectrum suggested the location of the steroid at C-2'. The HMBC spectrum of **5** showed interactions of C-3 with H-4 and H-1'; C-7 with H-6, H-5 and H_2-11; C-3'' with H-2', H_2-2'' and H_2-4''; and C-5'' with H_2-4'' and H-6''. Acid hydrolysis of **5** yielded D-glucose and *β*-sitosterol, TLC comparable. On the basis of spectral data analyses and chemical reactions the structure of **5** was formulated as 2-hydroxy-7-hydroxymethylene xanthone-1,8-dicarboxylic acid 3-*O*-*β*-D-glucopyranosyl-(2'→3'')-3''-*O*-stigmast-5-ene (Fig. 1). This is a new xanthonyl glucosidic sterol.

Compound **3** was the known steroidal glucoside *β*-sitosterol-*β*-*O*-glucoside.

All four new compounds were effective against the selected fungal strains at all concentrations tested. Compound **2** was effective against both *A. flavus* and *C. albicans* at the lowest tested concentration of 25 *μ*g/ml. It showed comparable results with those of the standard at higher concentration against *A. flavus* but in case of *C. albicans* the activity was lower than that of the standard drug. Compound **1** was found to be ineffective against *A. flavus* at the lowest tested concentration of 25 *μ*g/ml but showed activity at higher concentrations comparable with that of the standard drug. However, it was found to be ineffective against *C. albicans* and *P. citrinum* at all tested concentrations. Compounds **4** and **5** were found to be active against all the tested fungal strains at all concentrations (Table I).

In conclusion, the findings of the present work have revealed that the isolated xanthones from the seeds of *R. coriaria* possess antifungal activity.

Acknowledgement

The authors are thankful to the Head, Regional Sophisticated Center, Central Drug Research Institute, Lucknow, India for recording spectral data of the compounds and to the University Grants Commission, New Delhi, India for financial support.

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