# Phytochemical Investigations on *Indigofera oblongifolia*

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(Received 6th December, 2005, revised 19th January, 2006)

**Summary:** Seven compounds have been isolated for the first time from *Indigofera oblongifolia* namely, β-sitosterol (**1**), 3β-O-(*E*)-coumaroyllupeol (**2**), *N*-acetylhomoveratrylamine (**3**), ursolic acid (**4**), 7-O-methyleriodictyol (**5**), β-sitosterol 3-O-β-D-glucopyranoside (**6**) and chrysoeriol 7-O-β-D-glucopyranoside (**7**), respectively. Their structures have been elucidated by spectroscopic techniques.

## Introduction

*Indigofera oblongifolia* Forsk belonging to the family Fabaceae, is widely distributed in Asia and Africa. Its leaves are used in folk medicine in urinary tract infection, urolithiasis, cough, skin infection and also possess antimicrobial activity [1,2]. No phytochemical work on this species has so far been reported. The medicinal importance of *I. oblongifolia* prompted us to carry out phytochemical investigations on this species. We now report the isolation and structure elucidation of β-sitosterol (**1**), 3β-O-(*E*)-coumaroyllupeol (**2**), *N*-acetylhomoveratrylamine (**3**), ursolic acid (**4**), 7-O-methyleriodictyol (**5**), β-sitosterol 3-O-β-D-glucopyranoside (**6**) and chrysoeriol 7-O-β-D-glucopyranoside (**7**), respectively, from the chloroform fraction.

## Results and Discussion

The methanolic extract of the whole plant showed strong toxicity in brine shrimp lethality test. It was subsequently divided into *n*-hexane, chloroform, ethylacetate, *n*-butanol and water soluble fractions. Out of these the chloroform soluble fraction showed major toxicity in brine shrimp lethality test. This fraction has, therefore, been subjected to a series of column and flash chromatographic techniques as described in the experimental to obtain seven known compounds reported for the first time from *Indigofera oblongifolia*. These could be identified as β-sitosterol (**1**), 3β-O-(*E*)-coumaroyllupeol (**2**), *N*-acetylhomoveratrylamine (**3**), ursolic acid (**4**), 7-O-methyleriodictyol (**5**), β-sitosterol 3-O-β-D-glucopyranoside (**6**) and chrysoeriol 7-O-β-D-glucopyranoside (**7**), respectively, on the basis of spectral data. All these compounds individually showed lower level of toxicity compared to the crude chloroform extract thereby indicating that the strong toxicity of the latter is due to combined effect of various constituents present therein.

## Experimental

### *General*

Flash chromatography (FC): silica gel 230-400 mesh. Column chromatography (CC): silica gel 70-230 mesh. TLC: pre-coated silica gel 60 $F_{254}$ plates. UV: detection at 254 nm and using ceric sulphate reagent. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR spectra: Hitachi-UV-3200 and Jasco-302-A spectrophotometers, respectively. $^1$H- and $^{13}$C-NMR spectra: Bruker spectrometers operating at 500, 400 MHz; chemical shifts δ in ppm relative to $SiMe_4$ as internal standard and coupling constants J in Hz. EIMS, FABMS, HREIMS, HRFABMS: Jeol JMS-HX-110 and JMS-DA-500 mass spectrometers, m/z: (rel. int.). The purity of the isolated compounds was checked on pre-coated high performance thin layer chromatography (HPTLC) plates of E. Merck.

### *Plant Material*

The whole plant of *Indigofera oblongifolia* was collected from District Malir Karachi, in October 2003 and identified by Dr. Surriya Khatoon, Plant

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Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

*Isolation*

The shade dried whole plant (15 kg) was extracted with MeOH (3 × 45L) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (350 g). It was suspended in water and successively extracted with *n*-hexane, chloroform, ethylacetate and *n*-butanol. The chloroform soluble fraction (39 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, ethylacetate, ethylacetate-methanol and methanol in increasing order of polarity. The fractions obtained from *n*-hexane-ethylacetate (8.7:1.3) were combined and chromatographed over silica gel using *n*-hexane-chloroform (7.7:2.3) to obtain *β*-sitosterol (**1**). The fractions obtained from *n*-hexane-ethylacetate (7.5:2.5) gave three major spots on TLC, were combined and flash chromatographed over silica gel using the same solvent system to obtain 3*β*-O-(*E*)-coumaroyllupeol (**2**), *N*-acetylhomoveratrylamine (**3**) and ursolic acid (**4**), respectively. The fractions obtained from *n*-hexane-ethylacetate (5.5:4.5) were combined and re-chromatographed over silica gel using *n*-hexane-ethylacetate (1:1) as eluent to obtain 7-O-methyleriodictyol (**5**). The fractions obtained from ethylacetate-MeOH (9:1) were combined and chromatographed over silica gel using chloroform-methanol (8.8:1.2) to obtain *β*-sitosterol 3-O-*β*-D-glucopyranoside (**6**) and chrysoeriol 7-O-*β*-D-glucopyranoside (**7**), respectively.

*β-Sitosterol (1)*

It crystallized from acetone (24 mg), m.p. 136–137°C; $[\alpha]_D$ – 35.4° (*c* 0.0025, $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3446, 3050, 1650; $^1$H-NMR ($CDCl_3$, 400 MHz) *δ*: 5.23 (1H, m, H-6), 3.32 (lH, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.2 Hz, Me-21), 0.84 (3H, t, *J* = 7.0 Hz, Me-29), 0.83 (3H, d, *J* = 6.5 Hz, Me-26), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.68 (3H, s, Me-18); HREIMS, *m/z:* 414.3861 (calcd. for $C_{29}H_{50}O$, 414.3849). The physical and spectral data showed complete agreement with those reported in the literature [3-5].

*3β-O-(E)-Coumaroyllupeol (2)*

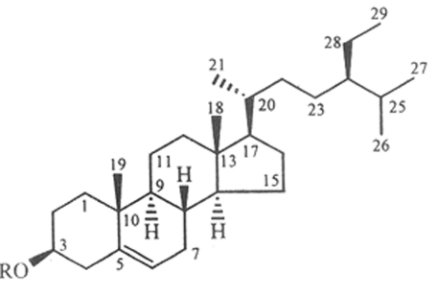
The fraction which eluted with *n*-hexane-ethylacetate (7.5:2.5) was flash chromatographed over silica gel eluting with the same solvent system collecting 150 fractions of 10 ml each. Compound **2** (16 mg) was obtained from fractions 30-50 as a crystalline solid, m.p. 82–85°C; $[\alpha]_D$ + 34° (*c* 0.001, $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3430, 1702, 1638; $^1$H-NMR ($CDCl_3$, 400 MHz) *δ*: 7.58 (1H, d, *J* = 15.9 Hz, H-3′), 7.41 (2H, d, *J* = 8.6 Hz, H-5′,9′), 6.81 (2H, d, *J* = 8.6 Hz, H-6′,8′), 6.28 (1H, d, *J* = 15.9 Hz, H-2′), 4.66, 4.57 (1H each, m, $CH_2$-29), 4.57 (1H, m, H-3), 1.66 (3H, s, Me-30), 1.03 (3H, s, Me-24), 0.95 (3H, s, Me-23), 0.93 (3H, s, Me-26), 0.90 (3H, s, Me-27), 0.88 (3H, s, Me-25) and 0.78 (3H, s, Me-28); HREIMS showed $[M]^+$ at *m/z* 572.4225 (calcd. for $C_{39}H_{56}O_3$, 572.4229). The physical and spectral data coincided with the literature values [6].

*N-Acetylhomoveratrylamine (3)*

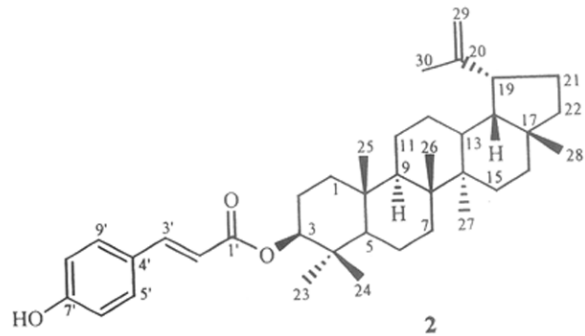
The fraction eluted with *n*-hexane-ethylacetate (7.5:2.5) was flash chromatographed over silica gel eluting with the same solvent system collecting 150 fractions of 10 ml each. Compound **3** (18 mg) was obtained from fractions 75-92 as colorless crystals, m.p. 104–105°C; $[\alpha]_D$ + 24° (*c* 0.0015, $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3440, 2925, 1054; $^1$H-NMR ($CDCl_3$, 400 MHz) *δ*: 6.85 (1H, dd, *J* = 8.1, 1.4 Hz, H-5′), 6.82 (1H, *d*, *J* = 1.4 Hz, H-2′), 6.74 (1H, d, *J* = 1.4 Hz, H-6′), 4.55 (1H, br s, N–H), 3.81, 3.78 (3H each, 2×OMe), 3.35 (2H, t, *J* = 7.4 Hz, H-1), 2.71 (2H, t, *J* = 7.4 Hz, H-2) and 1.89 (3H, s, MeCO); HREIMS showed $[M]^+$ at *m/z* 223.1201 (calcd. for $C_{12}H_{17}NO_3$, 223.1208). The physical and spectral data resembled the reported values [7].

*Ursolic acid (4)*

The fraction eluted with *n*-hexane-ethylacetate (7.5:2.5) was flash chromatographed over silica gel and eluted with the same solvent system collecting 150 fractions of 10 ml each. Compound **4** (16 mg) was obtained from fractions 110-122 as an amorphous powder, m.p. 291–292°C, $[\alpha]_D$ + 66.2° (*c* 0.011, $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3510, 3050, 1697, 1635, 820; $^1$H-NMR ($CDCl_3$, 400 MHz) *δ*: 5.11 (1H, m, H-12), 3.19 (1H, dd, $J_{ax,ax}$ = 10.0 Hz, $J_{ax,eq}$ = 4.5 Hz, H-3*α*), 1.20 (3H, s, Me-27), 1.07 (3H, s, Me-23), 0.94 (3H, s, Me-25), 0.91 (3H, d, *J* = 6.6 Hz Me-30), 0.86 (3H, s, Me-26), 0.81 (3H, s, Me-24), 0.80 (3H, d, *J* = 6.8 Hz, Me-29); HREIMS, *m/z:* 456.3603 (calcd. for $C_{30}H_{48}O_3$, 456.3595). The physical and spectral data showed complete resemblance with the reported values [8,9].



**1** R = H
**6** R = β-D-Glucose



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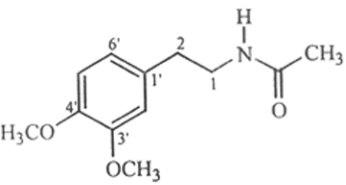

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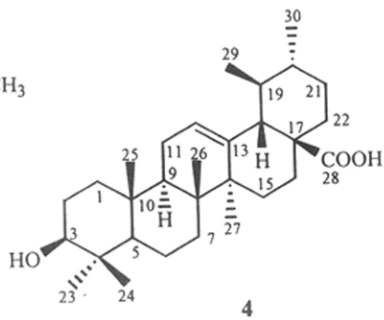

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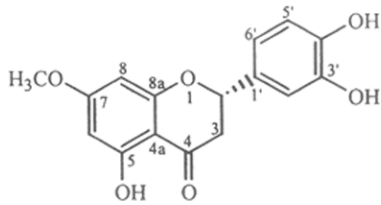
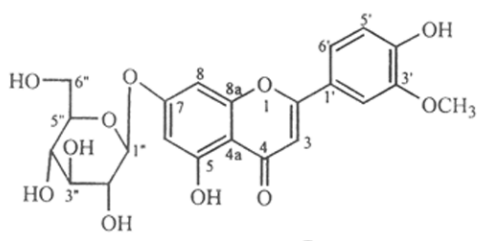


**5**



**7**

*7-O-Methyleriodictyol (5)*

The fractions obtained from *n*-hexane-ethylacetate (5.5:4.5) were combined and chromatographed over silica gel using *n*-hexane-ethylacetate (1:1) to obtain 7-O-methyleriodictyol **5** (24 mg) which crystallized from acetone, m.p. 221–222°C; $[\alpha]_D$ + 68.5° (*c* 0.001, $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3446,3050, 1650; $^1$H-NMR ($CDCl_3$, 400 MHz) *δ*: 7.26 (1H, d, *J* = 1.5 Hz, H-6), 7.23 (1H, d, *J* = 8.0 Hz, H-5'), 7.15 (1H, d, *J* = 1.5 Hz, H-8), 7.04 (1H, dd, *J* = 8.0, 1.4 Hz, H-6'), 6.46 (1H, d, *J* = 1.4 Hz, H-2'), 5.48 (1H, dd, *J* = 12.7, 2.6 Hz, H-2), 3.24 (1H, dd, *J* = 16.2, 12.7 Hz, H-3) and. 2.93 (1H, dd, *J* = 16.2, 2.6 Hz, H-3); HREIMS: *m/z*, 302.0780 (calcd. for $C_{16}H_{14}O_6$, 302.0790). The physical and spectral data showed complete agreement with those reported in the literature [10].

*β-Sitosterol 3-O-β-D-glucopyranoside (6)*

The fractions obtained from ethylacetate-MeOH (9:1) were combined and chromatographed over silica gel using chloroform-methanol (8.8:1.2) to obtain *β*-sitosterol 3-O-*β*-D-glucopyranoside **6** (22 mg) from the head fractions as a colorless amorphous powder, m.p. 232–235°C, $[\alpha]_D$ – 45° (*c* 0.023, MeOH); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3452, 3044, 1648. $^1$H-NMR ($CDCl_3$+$CD_3OD$, 400 MHz) *δ*: 5.13 (1H, m, H-6), 4.57 (1H, d, *J* = 7.3 Hz, H-1'), 3.85 (1H, m, H-3),

3.24-3.80 (m, Glc-H), 1.01 (3H, s, Me-19), 0.92 (3H, d, $J$ = 6.1 Hz, Me-21), 0.83 (3H, t, $J$ = 7.0 Hz, Me-29), 0.83 (3H, d, $J$ = 6.4 Hz, Me-26), 0.81 (3H, d, $J$ = 6.4 Hz, Me-27) and 0.68 (3H, s, Me-18); HRFABMS, *m/z:* 577.4444 $[M+H]^+$ (calcd. for $C_{35}H_{61}O_6$, 577.4438). The physical and spectral data were in agreement with the literature [3].

*Chrysoeriol 7-O-β-D-glucopyranoside (7)*

The fractions obtained from ethylacetate-MeOH (9:1) were combined and chromatographed over silica gel using chloroform-methanol (8.8:1.2) to obtain chrysoeriol 7-O-β-D-glucopyranoside 7 (14 mg) from tail fractions as a colorless amorphous powder, m.p. 176–179°C, $[\alpha]_D$ – 35° ($c$ 0.05, MeOH); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3452, 3044, 1648; $^1$H-NMR ($CDCl_3$+$CD_3OD$, 400 MHz) $\delta$: 7.54 (1H, dd, $J$ = 8.3, 1.3 Hz, H-6'), 7.50 (1H, d, $J$ = 1.3 Hz, H-2'), 6.93 (1H, d, $J$ = 8.3 Hz, H-5'), 6.84 (1H, d, $J$ = 1.6 Hz, H-6), 6.68 (1H, s, H-3), 6.49 (1H, d, $J$ = 1.6 Hz, H-8) and 5.06 (1H, d, $J$ = 7.0 Hz, H-1"); HRFABMS, *m/z:* 463.1233 $[M+H]^+$ (calcd. for $C_{22}H_{23}O_{11}$, 463.1240). The physical and spectral data showed complete agreement with the reported values [11,12].

## References


1. N. A. A. Ali, W. D. Julich, C. Kusnick and U. Lindequist, *J. Ethanopharmacology,* **74**, 173 (2001).
2. M.U. Dahot, *J. Ethanopharmacology,* **64**, 277 (1999).
3. A. A. Bernard and L. Eokes, *J. Org. Chem.,* **42**, 725 (1977).
4. I. Rubinstein, L. J. Goad, A. D. H. Clague and J. L. Mulheirw, *Phytochemistry,* **15**, 195 (1976).
5. H. L. Holland, P. R. P. Diakow and G. J. Taylor, *Can. J. Chem.,* **56**, 3121 (1978).
6. M. Au, A. Heaton, and D. Leach, *J. Nat. Prod.,* **60**, 1150 (1997).
7. A. Karimov, *Khim. Prir. Soedin,* **29**, 424 (1993).
8. M. Hadaka, M. Ito, Y. Matsuda, H. Kohda, K. Yamasak and J. Yamahora, *Phytochemistry,* **26**, 2023 (1987).
9. H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Am. Chem. Soc.,* **85**, 3688 (1963).
10. A. G. Gonzalez, J. Bermejo, F. Estevezn and R. Velazqez, *Phytochemistry,* **22**, 1515 (1983).
11. N. S. Kattaev, *Khim. Prir. Soedin,* **9**, 115 (1973).
12. W. A. Dement and T. J. Mabry, *Phytochemistry,* **2**, 1515 (1983).