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A New Alkaloid from *Isatis costata*

Itrat FATIMA[1], Itrat ANIS[1], Ijaz AHMAD[2], Abdul MALIK[1]*, Nighat AFZA[3]
[1]*International Center for Chemical Sciences, Hej Research Institute of Chemistry, University of Karachi, 75270, Karachi-PAKISTAN*
e-mail: abdul.malik@iccs.edu
[2]*Medicinal Botanic Centre, Pcsir Labs. Complex Peshawar, Peshawar, N.W.F.P, PAKISTAN*
[3]*Pharmaceutical Research Centre, Pcsir Labs. Complex, 75270, Karachi-PAKISTAN*

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A new alkaloid has been isolated from *Isatis costata* C.A.Mey. and assigned structure **1** on the basis of spectroscopic data including 1D and 2D NMR techniques. Methyl 2-acetoamidobenzoate (**2**), β-sitosterol (**3**), and ursolic acid (**4**) were also isolated for the first time from this species.

Key Words: *Isatis costata*, Brassicaceae, 2D NMR, structure elucidation.

Introduction

The genus *Isatis*, belonging to the family Brassicaceae, comprises 50 species mainly distributed in the Irano-Turanian region. In Pakistan, it is represented by 7 species.[1] *Isatis tinctoria*, also known as woad, is a common plant cultivated for centuries to produce the blue dye indigo. Nowadays, woad is also used in Chinese folk and modern medicine.[2] "Ban-Lan-Gen" is one of the most commonly used traditional Chinese medicines for antipyretic, anti-inflammatory, antiviral, and detoxifying purposes. Its original source was considered to be the dried roots of 3 plants, *Isatis indigotica*, *Isatis tinctoria*, and *Strobilanthes cusia*.[3,4] Now the roots of *Isatis indigotica* have been identified as the main source of "Ban-Lan-Gen" and recorded in Chinese Pharmacopoeia (1990 edn).[5] The ethnopharmacological importance of the genus *Isatis* prompted us to investigate the chemical constituents of *Isatis costata* C.A.Mey., which is an annual or biennial herb found in northern Pakistan. Previously 2 oxindole alkaloids have been reported from this species.[6] Herein we report the isolation and structural elucidation of a new alkaloid (**1**) along with methyl 2-acetoamidobenzoate (**2**), β-sitosterol (**3**), and ursolic acid (**4**), which are reported for the first time from this species.

Experimental

General experimental procedures

Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. IR spectra were measured on a JASCO 302-A spectrophotometer in $CHCl_3$. UV spectra were obtained on a Hitachi UV-3200 spectropho-

*Corresponding author

tometer. NMR spectra were run on a Bruker instrument. Chemical shifts δ are shown in ppm relative to TMS as internal standard and coupling constants J are described in Hz. EI, FAB, and HREI-MS were recorded on JEOL JMS-HX-110 and JMS-DA-500 mass spectrometers. Silica gel 230-400 mesh (E. Merck) was used for column chromatography. Silica gel plates (Si 60 F_{254}, E. Merck) were used for TLC.

Plant material

The whole plant material was collected in April 2004 from the Swat Valley in the Northwestern Frontier province of Pakistan and identified as *Isatis costata* C.A.Mey. by Dr. Ghosia Lutfullah, Center of Biotechnology, University of Peshawar, Pakistan. A voucher specimen (BPU-105) is deposited in the herbarium of the Department of Botany, University of Peshawar, Peshawar, Pakistan.

Extraction and isolation

The shade-dried whole plant (30 kg) was chopped and extracted 3 times with EtOH (60 L) at room temperature for 96 h. The ethanolic extract was evaporated in vacuo to give a dark greenish residue (400 g), which was partitioned between *n*-hexane and water. The aqueous fraction was further extracted with EtOAc and *n*-BuOH. The EtOAc fraction (12 g) was subjected to column chromatography eluting with *n*-hexane-EtOAc in increasing order of polarity to give 3 fractions. The silica gel column chromatography of the fraction obtained from *n*-hexane-EtOAc (7:3) showed 2 major spots on TLC, which on further purification by column chromatography over silica gel using *n*-hexane-EtOAc (8:2) as eluent yielded compounds **1** (11 mg) and **2** (15 mg). On the other hand, the eluent obtained from *n*-hexane-EtOAc (4:6) was rechromatographed over silica gel to afford 2 successive fractions using solvent system *n*-hexane-EtOAc (8:2-5:5). The second fraction was rechromatographed and eluted with *n*-hexane-EtOAc (7:3) to afford compounds **3** (9 mg) and **4** (11 mg) from the top and the tail fractions, respectively.

Compound 1: White amorphous solid, mp 110-112 °C; UV (MeOH) λ_{max} nm (log ε) = 205 (4.08), 288 (3.25), 175 (4.12). IR (KBr) ν_{max} = 3440, 1717, 1690, 1680, 1615, 1500, 1455 cm^{-1}. EI-MS, m/z (%) = 331 [M^+] (47), 224 (100), 120 (72), 119 (40), 91 (45), 77 (85). HRFABMS m/z 331.1208 (calc. for $C_{21}H_{17}NO_3$ 331.1205). Complete assignments of ^{13}C- and ^{1}H-NMR signals for **1** are described in the Table. Important HMBC correlations are illustrated in the Figure.

Methyl 2-acetoamidobenzoate (2): White amorphous solid, mp 101 °C; IR (KBr) ν_{max} = 1690, 1680, 1615, 1500, 1455, 3440 cm^{-1}. HRFABMS m/z 193.0738, (calc. for $C_{10}H_{11}NO_3$193.0735). EI-MS, ^{13}C- and ^{1}H-NMR data were identical to those reported in the literature.[7]

***β*-Sitosterol (3):** Colorless crystals, mp 258-262 °C; IR (KBr), ν_{max} = 3450, 3050, 1650, 81 cm^{-1}; EI-MS and NMR data were identical to those reported in the literature.[8]

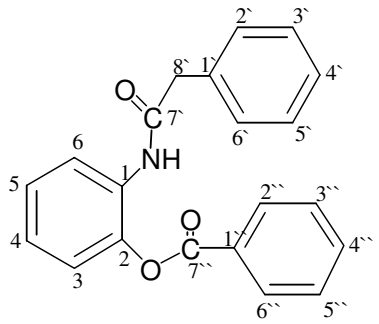
Ursolic acid (4): Colorless crystals, mp 110-112 °C; IR (KBr), ν_{max} = 3510, 3050, 1697, 1635, 820 cm^{-1}. EI-MS and NMR data were identical to those reported in the literature.[9]

Results and Discussion

The ethanolic extract of shade-dried whole plant (30 kg) of *Isatis costata* C.A.Mey. was partitioned in between *n*-hexane, EtOAc, *n*-BuOH, and H_2O. As a result of a series of column chromatographic techniques compounds **1-4** were isolated from the EtOAc fraction as described in the Experimental part.

Table. ^{13}C- (125 MHz) and ^{1}H-NMR (500 MHz) spectral data (CD_3OD) for compound **1**.

Position	^{13}C (δ, ppm)	^{1}H (Pδ, ppm)
1	120.8	-
2	139.3	-
3	127.5	7.78, dd, J = 8.3,0.8 Hz
4	129.3	7.55, ddd, J = 8.3, 7.6, 1.5 Hz
5	122.9	7.09, ddd, J = 8.4, 7.6, 0.8 Hz
6	121.3	8.78, dd, J = 8.4, 1.5 Hz
1′	137.6	-
2′	128.6	7.35, m
3′	129.1	7.35, m
4′	131.3	7.35, m
5′	129.1	7.35, m
6′	128.6	7.35, m
7′	169.1	-
8′	43.6	4.54, d, J = 5.5 Hz
1″	134.4	-
2″	127.4	7.98, dd, J = 7.5, 1.4 Hz
3″	128.5	7.48, m
4″	132.4	7.46, m
5″	128.5	7.48, m
6″	127.4	7.98, dd, J = 7.5, 1.4 Hz
7″	165.9	-
N–H	-	12.1



Compound 1

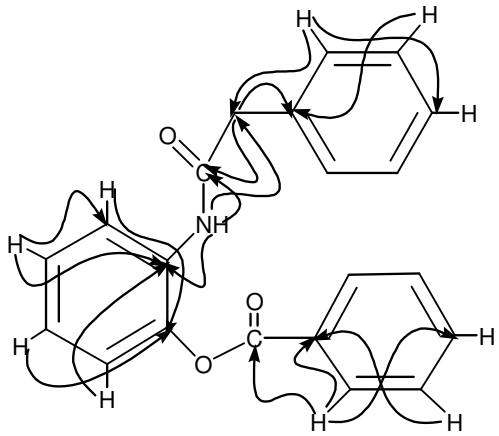


Figure. The significant long range correlations detected in the HMBC spectrum of **1**.

Compound **1** was isolated as a white amorphous solid and gave positive test results with Dragendorff's reagent for nitrogenous compounds. The molecular ion peak at m/z 331.1201 in the HR-EIMS indicated its molecular formula to be $C_{21}H_{17}NO_3$ (calc. 331.1205). The UV spectrum in MeOH showed λ_{max} at 205, 288, and 175 nm. Absorption bands in the IR spectrum of **1** suggested the presence of an amide (3440 cm^{-1}), carbonyl functionalities (1690, 1680 cm^{-1}), and an aromatic ring system (1615, 1500, 1455 cm^{-1}).

In the ^{1}H-NMR 14 aromatic protons were observed and could be assigned to a 1,2 disubstituted benzene and a pair of monosubstituted phenyl rings on the basis of ^{1}H-^{1}H COSY spectrum and coupling patterns. The ^{1}H-NMR displayed a dd at δ 8.78 (J = 8.4, 1.5 Hz, H-6), dd at δ 7.78 (J = 8.3, 0.8 Hz, H-3), and a pair of ddd at δ 7.55 (J = 8.3, 7.6, 1.5 Hz, H-4) and δ 7.09 (J = 8.4, 7.6, 0.8 Hz, H-5), respectively, confirming the presence of a 1,2 disubstituted benzene ring. It also showed a pair of monosubstituted phenyl rings [multiplets at δ 7.35 (5H) and dd of H-2″ and 6″ at δ 7.98 (J = 7.5, 1.4 Hz), 7.46 (1H), 7.48 (2H)], respectively. The signals at δ 4.54 (2H, d, J = 5.5 Hz) and 12.1 (1H, brd) could be assigned to benzylic and amide protons, respectively.

The ^{13}C-NMR spectrum (BB and DEPT) showed 21 carbon signals, in which there were 1 methylene, 14 methine, and 6 quaternary carbons. The low-field region of the ^{13}C spectrum showed 2 signals at δ 165.9 (C-7″) and 169.1 (C-7′), which could be assigned to the amide and ester moieties. The methylene carbon was observed at δ 43.6. The signals at δ 120.8-139.3 were due to aromatic carbons.

In the HMBC spectrum (Figure), the benzylic protons (H-8′, δ 4.54) showed ^2J correlations with C-1′ (δ 137.6) and C-7′ (δ 169.1). The amide proton (NH, δ 12.1) showed ^2J correlations with C-1 (δ 120.8) and C-7′ (δ 169.1), and a ^3J correlation with C-8′ (δ 43.6), thereby providing conclusive evidence for the attachment of an amide group to the benzyl and substituted phenyl moieties through the intervening carbonyl group. The H-2′ at δ 7.35 showed ^3J correlations with C-8′ (δ 43.6) and C-4′ (δ 131.3), while the H-3′ at δ 129.1 showed a ^3J correlation with C-1′ (δ 137.6). The H-6″ (δ 7.98) showed a ^2J correlation with C-1″ (δ 134.4) and ^3J correlations with C-7″ (δ 165.9) and C-4″ (δ 132.4). The H-5″ at δ 7.48 showed a ^3J correlation with C-1″ (δ 134.4), while H-5 at δ 7.09 showed a ^2J correlation with C-6 (δ 121.3) and a ^3J correlation with C-1 (δ 120.8). The H-6 at δ 8.78 showed ^3J correlation with C-2 (δ 139.3) and the H-3 at δ 7.78 showed a ^3J correlation with C-1 (δ 120.8). On the basis of these data, compound **1** was assigned the structure 2-[(2-phenylacetyl) amino] phenyl benzoate.

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