Journal of Enzyme Inhibition and Medicinal Chemistry, June 2008; 23(3): 386–390

informa
healthcare

Urease inhibitor from *Datisca cannabina* linn

MANSOOR AHMAD[1], NOOR MUHAMMAD[1], MANZOOR AHMAD[2], MUHAMMAD ARIF LODHI[2], MAHJABEEN [1], NOOR JEHAN[1], ZAHID KHAN[1], ROSA RANJIT[2], FARZANA SHAHEEN[2], & MUHAMMAD IQBAL CHOUDHARY[2]

[1]*Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan, and* [2]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*

(*Received 23 April 2007; accepted 25 May 2007*)

Abstract
A new flavonoid datisdirin (**1**), along with eight known compounds tectochrysine (**2**), cearoin (**3**), sideroxyline (**4**), ursolic acid (**5**), corosolic acid (**6**), arjunolic acid (**7**), erythrodiol (**8**) and oleanolic acid (**9**), were isolated from the ethyl acetate fraction of *D. cannabina* Linn. The structure of compound **1** was deduced on the basis of its spectral data. Datisdirin showed activity against the ureases enzyme.

Keywords: *Urease, inhibition,* Datisca cannabina*, datisdirin*

Introduction

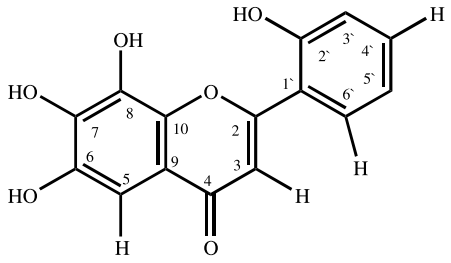
Datisca cannabina (Datiscaceae) is a glabrous herb, usually grows in light (sandy), medium (loamy), heavy (clay), acidic, neutral and basic and well-drained soils. It requires moist soil but cannot grow in the shade [1]. The plant is distributed in tropical and subtropical western Himalaya, from Kashmir to Nepal, Turkey, Afghanistan, and Pakistan [2]. It is bitter in taste and purgative in nature. The whole herb used as medicine for fevers, gastric and scrofulous (or scrofula, diseased run-down appearance) complaints [3]. Medicinally, it acts as a sedative in rheumatism and used as an expectorant in catarrh, also used locally to carious teeth [1].

Activity of ureases (E.C 3.5.1.5) has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which is detrimental for human and animal health as well as for agriculture. Urease is directly involved in the formation of stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia, hepatic encephalopathy, hepatic coma, urinary catheter encrustation [4–5]. It is also known to be a major cause of pathologies induced by *Helicobacter pylori* (HP), which allows bacteria to survive at low pH of the stomach during colonization and therefore, plays an important role in the pathogenesis of gastric and peptic ulcer (including cancer) [5]. In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. This further induces plant damage primarily by depriving them from their essential nutrient and secondly ammonia toxicity, which increases the pH of the soil [6–7]. Therefore strategies based on urease inhibition are now considered as the first line of treatment for infections caused by urease producing bacteria.

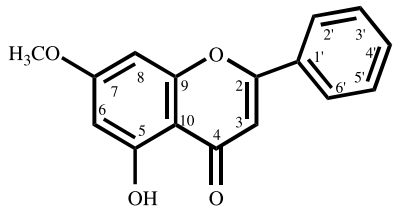
Our detail investigation on *D. cannabina*, has yielded one new compound datisdirin (1), along with eight known compounds. Among known compounds, six were tectochrysine (2) [8], cearoin (3) [9], sideroxyline (4) [10], ursolic acid (5) [11], corosolic acid (6) [12], and arjunolic acid (7) [13], which is reported for the first time from this genus and two

Correspondence: Prof. Dr. Mansoor Ahmad, Institute of Pharmaceutical Sciences, University of Karachi, Karachi 75270, Pakistan. Tel.: +92-21-9261300-7. Ext. 2322. Fax: +92-21-9261340. E-mail: herbalist51@yahoo.com

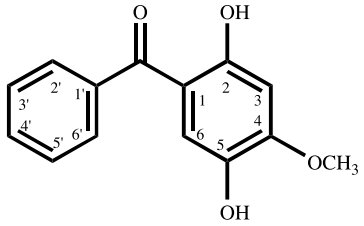
ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360701536513



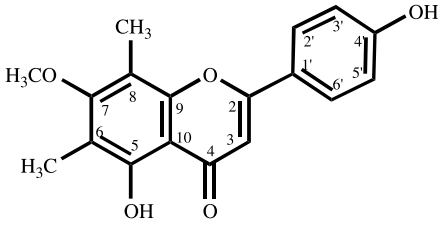
Structure of compound **1**



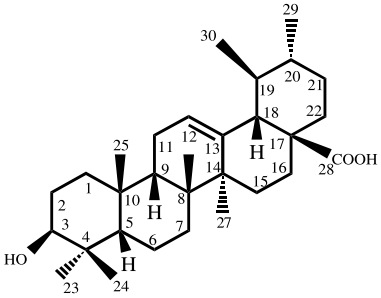
Tectochrysine (**2**)



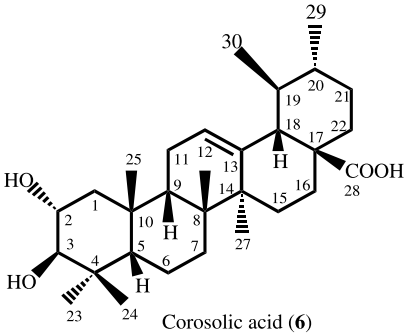
Cearoin (**3**)



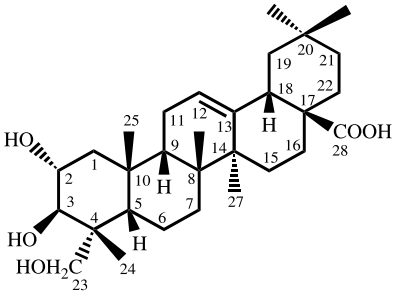
Sideroxyline (**4**)



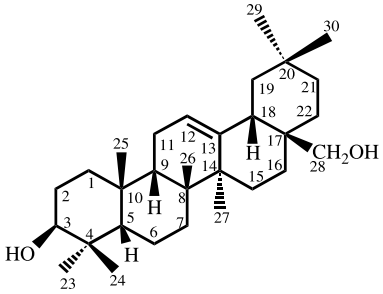
Ursolic acid (**5**)



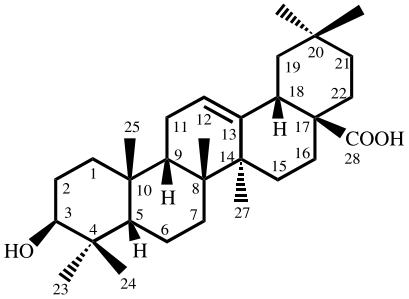
Corosolic acid (**6**)



Arjunolic acid (**7**)



Erythrodiol (**8**)



Oleanolic acid (**9**)

triterpen erythrodiol (8) [14], and oleanolic acid (9) [15] were previously reported from this plant [16]. The structure of the known compounds 2–9 were confirmed by comparing with literature data.

Experimental

General

Optical rotations were measured on a JASCO DIP 360 polarimeter. IR spectra were recorded on a JASCO 302-A spectrophotometer. EI-MS and HREI-MS were recorded on Jeol JMS HX 110 with data system and on JMS-DA 500 mass spectrometers. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker NMR spectrometers, operating at 500 and 400 MHz (100 and 125 MHz for ^{13}C). The chemical shifts values are reported in ppm (δ) units and the coupling constants ($\mathcal{J}$) are given in Hz.

Chromatographic conditions

For thin layer chromatography (TLC) precoated aluminium sheets (silica gel G-60F-254, E. Merck) were used. Visualization of the TLC plates was achieved under ultra-violet (UV) at 254 and 366 nm and by spraying with cerric sulphate reagent. Solvent system *n*-hexane- ethyl acetate (7:2, 9.5:0.5) was used.

Plant material

Whole plant of *D. cannabina* Linn., was collected from Matta, Swat District, N.W.F.P. Pakistan, during September 2001, and was identified by Mr. Mehboob-ur-Rehman, Assistant Professor, Jahan Zeb Post Graduate College, Saidu Sharif, Swat (Pakistan). A voucher specimen (CD-01/2001) was deposited in the herbarium of the Department of Botany.

Extraction and isolation

The freshly collected air-dried powdered plant material (15.0 kg), were percolated in 80% ethanol (3 × 20 L) at room temperature for 15 days. The percolate was filtered through Whatman filter paper. The combined ethanol extract was evaporated and the concentrated viscous extract (620 g) was consecutively partitioned between *n*-hexane, ethyl acetate and butanol. The ethyl acetate fraction (20 g) was subjected to column chromatography over a silica gel column (500 g, 70–230 mesh, E Merck) using *n*-hexane with gradient of ethyl acetate up to 100% and methanol up to 20% as eluent. Eighteen fractions were collected. The initial fractions, which were prominent in hexane, contained mostly triterpenes, where as the ethyl acetate fractions contained mostly flavonoids. The flavonoids were isolated using repeated column chromatography (flash silica gel, 230–400 mesh), preparative TLC (silica gel 60 GF254) using different ethyl acetate-hexane systems. Sub fraction 12 (1.2 g) obtained from first column was loaded on a silica gel column using a system of Hexane-EtOAc (50:50) yielded compound 1 (10 mg), cearoin (6.2 mg), sideroxyline (4.3 mg) and tectochrysine (7.5 mg). Sub fraction 6 (290 mg) of the first column was loaded on silica gel (flash silica 230–400 mesh) and eluted with 40% ethyl acetate-hexane, from which five compounds oleanolic acid (6.3 mg), erythrodiol (4.8 mg), ursolic acid (6.5 mg), corosolic acid (7.0 mg), and arjunolic acid (8.4 mg), were isolated.

Datisdirine (2′,6,7,8-Tetrahydroxyflavone) (1). Yellow amorphous powder (10 mg); Melting Point: 248–250°, IR $CHCl_3$ $\nu_{max}cm^{-1}$:3448 (OH), 2968 (aromatic CH), 1715 (C=O), 1645, 1265, and 1120; UV (MeOH) λ_{max}: 275, 252 nm, HRMS (m/z):286.2364 (calculated); 286.2361 (rel-wt); ^{1}H, ^{13}C-NMR δ (see Table I).

Urease assay and inhibition

Reaction mixtures comprising 25 μL of enzyme (Jack bean urease) solution and 55 μL of buffers containing 50 mM urea were incubated with 5 μL of test compounds (1 mM concentration) at 30°C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [16]. Briefly, 45 μL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min.) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (50 mM $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula {100 − (ODtestwell/ODcontrols) × 100)}. Thiourea was used as the standard inhibitor of urease.

Results and discussion

Datisdirin (1) was isolated from the ethyl acetate fraction of the crude extract of *Datisca cannabina* Linn. as yellow amorphous powder having melting point

Table I. ^{1}H and ^{13}C-NMR data of Datisdirin (1) in $CDCl_3$.

C-atoms	^{1}H-NMR δ ($\mathcal{J}$ in Hz)	^{13}C-NMR (δ)	Multiplicity	HMBC correlation
2		163.5	C	
3	6.4, *br s*	97.7	CH	2, 4, 9
4		179.1	C	
5	6.17, *br s*	93.3	CH	4, 9, 6
6		156.8	C	
7		160.5	C	
8		143.4	C	
9		103.1	C	
10		146.8	C	
1′		120.8	C	
2′		158.4	C	
3′	6.77, d, $\mathcal{J}$ = 8.1 Hz	118.9	CH	2′, 4′, 3′, 5′
4′	7.23, t, $\mathcal{J}$ = 7.2 Hz	130.6	CH	3′, 5′, 6′, 2′
5′	6.83, t, $\mathcal{J}$ = 7.1 Hz	117.8	CH	2′, 3′, 4′, 6′, 1′
6′	7.67, d, $\mathcal{J}$ = 7.7 Hz	127.6	CH	1′, 2′, 4′, 5′

248–250°C. Its characteristic yellow color on TLC with cerric sulphate spray and heating identified as flavonoid.

The UV spectrum displayed the λ_{max} at 275 and 252 nm. These values also indicated that the nature of compound **1** is a flavone. The IR spectrum of compound **1** showed absorption band at 3448 (OH), 2968 (aromatic CH), 1715 (C=O), 1645, 1265, 1120 cm^{-1}.

The HREI-MS exhibited a molecular ion at *m/z* 286.2361, (286.2364 calcd); with the agreement of molecular formula $C_{15}H_{10}O_6$. In ^{1}H-NMR (Table I) the characteristic aromatic protons for the flavone resonated at δ 6.77 (d, $\mathcal{J}$ = 8.1 Hz) assigned to H-3′, 7.23 (t, $\mathcal{J}$ = 7.2 Hz) to H-4′, 6.83 (t, $\mathcal{J}$ = 7.1 Hz) to H-5′ and 7.67 (d, $\mathcal{J}$ = 7.7 Hz) to H-6′., which indicated the ortho substitution on ring B.

The ^{13}C–NMR spectra (BB, DEPT) (Table I) of compound **1** revealed the presence of fifteen carbon signals, including six methine and nine quaternary carbons. A downfield signal at δ 179.1 assigned to carbonyl carbon, C-4. This was supported by two prominent fragments at *m/z* 118 and 168 obtained of Retro Diels Alder fragmentation of the heterocyclic ring C at EIMS [17]. Similarly these ions confirmed the ring A was substituted by three hydroxyl groups and B with one hydroxyl group. The ^{1}H-^{13}C correlations were determined by HMQC and long-range ^{1}H-^{13}C connectivity HMBC techniques (Figure 1). In the HMBC spectrum the singlet at δ 6.17 showed cross peak with the carbonyl carbon (δ 179.1), thereby assigning its position to C-5. The rest of HMBC correlations are in complete agreement to the assigned structure. On the basis all the spectroscopic data deduced the structure of compound **1** as flavone and having 2′, 6, 7, 8-tetrahydroxyflavone.

Certain synthetic classes of compounds like hydroxamic acids, imidazoles and phosphazenes have shown potential urease inhibition [18]. Unfortunately, no natural product with such activity has been discovered so far. So, there was a need to search for inhibitors of urease from natural resources. Compound 1 was tested against the specified enzyme and showed potent activity (IC_{50} 83.79 ± 0.023 μM, standard thiourea IC_{50} 21.01 ± 0.51 μM).

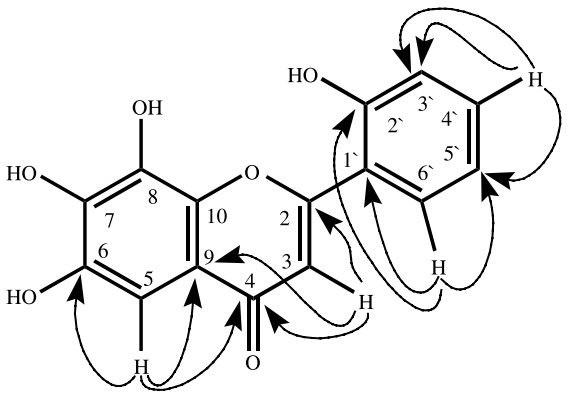


Figure 1. Selective HMBC interactions in compoud **1**.

References

[1] Kirtikar KR. Indian Medicinal Plants. Allahabad Bhuwaneswari Bahadur Ganj Allahabad: Indian Press Panni Office; 1918. p 610.

[2] Nasir E, Ali SI. Flora of West Pakistan. Karachi: Feroz Sons Press; 1973.

[3] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research; 1986.

[4] Schwabe W. Ger. Offen. 2, 159, 923 (Cl. A 61K), 1973, Appl. P 21 59 923.5–41. 1971;16.

[5] Kupchan SM, Tsou G, Sigel CW. J Org Chem 1973;38(7): 1420–1421.

[6] Pangarova TT, Zapesochnaya GG. Khim Prir Soedin 1974; 10(6):788–789.

[7] Pangarova TT, Zapesochnaya GG. Khim Prir Soedin 1974; 10(6):790–791.

[8] Lounasmaa M, Puri HS, Widen CJ. Phytochemistry 1977;16: 1851.
[9] De Souza GIS, Gotlieb OT, Souza ACH, Magalhaes MT. Phytochemistry 1975;14:1452–1453.
[10] Hillis WE, Koichiro I. Phytochemistry 1965;4:541–550.
[11] Lounasmaa M, Widen CJ, Reichstein T. Helv Chem Acta 1971;54:2850.
[12] Sakakibara J, Kaiya T, Fukunda H, Ohki T. Phytochemistry 1983;22:2553.
[13] Furuya T, Orihara Y, Hayashi C. Phytochemistry 1987;26:715.
[14] King FE, King TJ, Ross JM. J Chem Soc 1954;3995.
[15] Xue H-Z, Lu Z-Z, Konno C, Soejarto DD, Cordell GA, Fong HHS, Hodgson W. Phytochemistry 1988;27:233.
[16] Sharma VK, Jain MP, Kaul SK, Rao PR. Indian J Pharm Sci 1980;42(1):24–25.
[17] Grisebach H, Grambow HJ. Phytochemistry 1968;7(1): 51–56.
[18] Pangarova TT, Zapesochnaya GG. Rastit Resur 1976;12(2): 237–241.