Industrial Utilization of Medicinal and Aromatic Plants Area of Earth[1], Environmental and Marine Sciences and Technologies, ICS-UNIDO, Trieste, Italy, Department of Forest Products[2], University of Horticulture and Forestry, Nauni-Solan, India, Department of Pharmacognosy and Phytochemistry[3], Niigata College of Pharmacy, Niigata, Japan and Phytopharmaceutical Research Laboratory[4], School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen, Scotland, U.K.

# 2′-(2,3-Dihydroxybenzoyloxy)-7-ketologanin: a novel iridoid glucoside from the leaves of *Gentiana kurroo*

V. Kumar[1], R. Chand[2], A. Auzi[1], Y. Ikeshiro[3], S. D. Sarker[4]

*Received February 19, 2003, accepted April 4, 2003*

*Dr. Satyajit D. Sarker, BPharm (Hons), MPharm, PhD, ILTM, Phytopharmaceutical Reserch Laboratory, School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen, AB10 1FR, UK*
*s.sarker@rgu.ac.uk*

*Pharmazie 58: 668–670 (2003)*

A new bitter acylated iridoid glucoside, 2′-(2,3-dihydroxybenzoyloxy)-7-ketologanin (**1**), has been isolated from the leaves of *Gentiana kurroo*. The structure of the compound was elucidated conclusively by chemical analysis, and extensive 1D and 2D NMR experiments.

## 1. Introduction

Indian Gentian, *Gentiana kurroo* Royle (Gentianaceae), is a well known medicinal plant and has been used in traditional Ayurvedic medicine as a popular remedy for intermittent fevers, acidity and its bilious dyspepsia, fever, functional inactivity of liver [1]. The roots of *G. kurroo* have previously been reported to contain bitter principles and yellow brittle transparent resin resembling mastic [2]. Later, a number of iridoid glycosides have been reported from the roots and rhizomes of this plant [3]. We now report on the isolation and identification of a new iridoid glucoside from the leaves of *G. kurroo*.

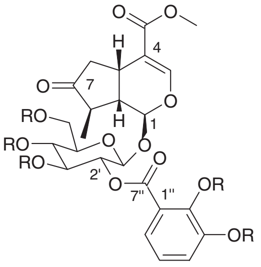
**Table 1: $^1$H- and $^{13}$C NMR data (400 and 100 MHz, respectively; δ-values in ppm, J in Hz in parenthesis) for 1**

| C/H | $\delta_H$ [a] | $\delta_C$ [a] | $^1$H-$^1$H-COSY | $^1$H-$^{13}$C correlations [b] | |
|---|---|---|---|---|---|
| | | | | $^2$J | $^3$J |
| 1α | 5.63 d (2.4) | 95.2 | H-9 | | C-3, C-5, C-1′ |
| 3 | 7.01 s | 152.1 | – | C-4 | C-1, C-5, C-11 |
| 4 | – | 111.3 | – | – | – |
| 5β | 3.03 bt (8.6) | 27.6 | H-6, H-9 | C-4, C-6, C-9 | C-3, C-7 |
| 6α | 2.40* | 42.9 | H-5 | C-5, C-7 | C-4 |
| 6β | 2.56 dd (8.0, 19.2) | | H-5 | C-5, C-7 | C-4, C-9 |
| 7 | – | 220.2 | – | | |
| 8α | 1.94 m | 44.2 | H-9 | C-9, C-10 | C-1, C-7 |
| 9β | 2.33* | 46.4 | H-1, H-9 10 (Me) | C-1, C-5, C-8 | C-3, C-10 |
| 10 (Me) | 1.11 d (7.2) | 13.1 | H-9 | C-8 | C-7, C-9 |
| 11 | – | 167.9 | – | – | – |
| COOMe | 3.30 s | 51.7 | – | – | C-11 |
| 1′ | 5.03 d (8.4) | 98.1 | H-2′ | C-2′ | C-1, C-3′ |
| 2′ | 5.01 dd (8.4) | 75.4 | H-1′, H-3′ | C-1′, C-3′ | C-7″ |
| 3′ | 3.73* | 75.5 | H-2′, H-4′ | C-2′ C-4′ | C-5′ |
| 4′ | 3.43 dd (8.4) | 71.7 | H-3′, H-5′ | C-3′, C-5′ | C-6′ |
| 5′ | 3.49* | 78.6 | H-4′, H-6′ | C-4′ | C-3′ |
| 6′ | 3.97 dd (2.0, 11.6) | 62.6 | H-5′ | | C-4′ |
| | 3.74* | | H-5′ | | C-4′ |
| 1″ | – | 113.6 | – | – | – |
| 2″ | – | 151.5 | – | – | – |
| 3″ | – | 147.0 | – | – | – |
| 4″ | 7.03 dd (1.2, 8.0) | 121.8 | H-5″, H-6″ | C-3″ | C-2″, C-6″ |
| 5″ | 6.75 dd (8.0, 8.0) | 120.0 | H-4″, H-6″ | C-4″ | C-1″, C-3″ |
| 6″ | 7.36 dd (1.2, 8.0) | 121.3 | H-4″, H-5″ | | C-2″, C-4″, C-7″ |
| 7″ | – | 170.6 | | | |

[a] Spectra obtained in $CD_3OD$ referenced to δ 3.31 ($^1$H) and δ 49.15 ($^{13}$C).
[b] $^1$H-$^{13}$C $^2$J and $^3$J correlations from HMBC experiment. * overlapped peaks

## 2. Investigations, results and discussion

The UV spectrum of **1** was indicative of a structure containing a phenolic moiety. The SIMS and EIMS spectra revealed the $[M+H]^+$ and $[M]^+$ ions, respectively, at *m/z* 525 and 524.47 corresponding to the molecular formula $C_{24}H_{28}O_{13}$. The $^1H$ and $^{13}C$ NMR spectra (Table 1), in addition to the signals associated with a 7-ketologanin skeleton [4], exhibited signals for a 2,3-dihydroxybenzoyl moiety. The signals for the oxymethine proton and carbon of C-2′ of **1** ($\delta_H$ 5.01 and $\delta_C$ 75.4) were more deshielded than those published for 7-ketologanin ($\delta_H$ 3.97–4.07 and $\delta_C$ 74.8). This deshielding effect indicated the presence of 2,3-dihydroxybenzoyl moiety at C-2′. This fact was confirmed further from the HMBC spectrum (Table 1) where a $^1H$-$^{13}C$ long-range ($^3J$) correlation from H-2′ to the benzoyl carbonyl was observed. The $^{13}C$ NMR spectrum (Table 1) displayed signals for all 24 carbons including a ketonic carbonyl (C-7), two ester carbonyls (C-11 and C-7″), olefinic methine (C-3) and olefinic quaternary (C-4), six oxymethines (one of them is for C-1 and the other five for the glucose unit C-1′ to C-5′), three methines (C-5, C-8 and C-9), one methylene, one oxymethylene (C-6′), two methyls (C-10 and C-11 acetate methyl), three aromatic methines (C-3″, C-4″ and C-5″) and three aromatic quaternaries (C-1″ to C-3″) two of which are oxygenated (C-2″ and C-3″). A 2D $^1H$-$^1H$ COSY spectrum (Table 1) revealed all possible spin systems within **1** which include correlations: H-1 ↔ H-9 ↔ H-5 ↔ $H_2$-6, H-1 ↔ H-9 ↔ H-8 ↔ $H_3$-10, $H_3$-10 ↔ H-8 ↔ H-5 ↔ $H_2$-6, H-1′ ↔ H-2′ ↔ H-3′ ↔ H-4′ ↔ H-5′ ↔ H-6′ and H-4″ ↔ H-5″ ↔ H-6″. While the HETCOR spectrum of **1** revealed all directly coupled $^1H$ and $^{13}C$ nuclei, the HMBC (Table 1) exhibited $^2J$ and $^3J$ $^1H$-$^{13}C$ long-range correlations in the molecule. The most notable HMBC correlations were: $^3J$ from H-1′ to C-1 and H-1 to C-1′ confirming the attachment of the glucose unit at C-1, and from H-2″ to C-7″ ($^3J$) which established that the 2,3-dihydroxybenzoyl unit is linked to C-2″. The relative stereochemistry at the chiral centres of **1** was established primarily by comparison of its $^1H$ and $^{13}C$ data with related molecules [4–6] and confirmed by a 2D $^1H$-$^1H$ NOESY experiment (Table 2). The acetylation of **1** produced a pentaacetate derivative which confirmed further the presence of three hydroxyl groups of the glucose unit and two of the benzoyl moiety. Thus the structure of the iridoid glucoside was elucidated conclusively as 2′-(2,3-dihydroxybenzoyloxy)-7-ketologanin (**1**) and, to our knowledge, it is a new natural product.



| | | |
|---|---|---|
| **1** | 2’-(2, 3-Dihydroxybenzoyloxy) 7-ketologanin | R = H |
| **2** | 2’-(2, 3-Dihydroxybenzoyloxy 7-ketologanin pentacetate | R = Ac |

Occurrence of iridoid glycosides, including loganin derivatives, is common in the genus *Gentiana* [3–8]. However, the site of glycosylation (normally C-1 or C-7) and the extent of acylation on the sugar unit vary considerably among the *Gentiana* species. Iridoid/secoiridoid glucosides have previously been used as chemotaxonomic markers in the Loasoideae [9], and their chemotaxonomic significance in the Gentianaceae has recently been discused by Jensen and Schripsema [3]. Therefore, co-occurrence of loganin-type iridoid glucosides among the *Gentiana* species certainly has some chemotaxonomic implications.

**Table 2: $^1H$-$^1H$ nOe interactions in 1, obtained from NOESY experiment.**

| From | To | From | To |
|---|---|---|---|
| H-1α | H-1′, H-6a, H-9β*, 10 (Me)† | H-1′ | H-1, H-3′, H-5′ |
| H-3 | – | H-2′ | H-4′ |
| H-5β | H-9β, H-6β | H-3′ | H-1′, H-5′ |
| H-6α | H-1′ | H-4′ | H-2′ |
| H-6β | H-5β, H-9β | H-5′ | H-1′, H-3′, H-6′* |
| H-8α | C-9β,* 10 (Me)* | H-6′ | H-5′* |
| H-9β | 10 (Me), H-5β, H-6β, H-8α* | H-4″ | H-5″ |
| 10 (Me) | H-9β, H-8α*, H-1α† | H-5″ | H-4″, H-6″ |
| COOMe | – | H-6″ | H-5″* |

Spectrum obtained in $CD_3OD$
* COSY-type correlation
† Weak correlation

## 3. Experimental

### 3.1. General

M.p. was determined using a sulphuric acid bath and was uncorrected. The UV spectrum was recorded on a Shimadzu-240 UV-Vis spectrophotometer. Silica gel G (60–120 mesh, Merck) and silica gel $GF_{254}$ (Merck) were used for CC and TLC, respectively. Spots on TLC plates were visualised under UV light (254 nm) and after exposure to iodine vapours. TLC of compounds was carried out using ethyl acetate : methanol : water (77 : 15 : 8) as the solvent system. Ms was obtained using a Hitachi M-2500 Mass Spectrometer and NMR experiments were performed on a JEOL JNMA 400 MHz NMR spectrometer. Tetramethylsilane (TMS) was used as an internal standard for NMR.

### 3.2. Plant material

Leaves of *Gentiana kurroo* Royle were collected from the Mangarh-Kainthli hill- tops of Sirmour district of Himachal Pradesh (India). A voucher specimen (No. 7138 dated 15-09-1998) for this collection has been deposited at the herbarium of University of Horticulture and Forestry, Solan (Himachal Pradesh) India.

### 3.3. Extraction and isolation

The dried and ground aerial parts (500 g) of *G. kurroo* was extracted with MeOH (2 l × 4) under reflux for 2 h. The combined MeOH extracts were concentrated on a water bath and finally dried under vacuum. The vacuum dried residue was refluxed (2 h) with benzene (200 ml × 4) and filtered to remove non-bitter principles. The benzene-insoluble residue was dried under vacuum. The residue was mixed with $H_2O$ (100 ml) and then extracted with EtOAc (200 ml × 4) using a separating funnel. The EtOAc layer was concentrated under vacuum and subjected to CC eluting with a mixture of $CHCl_3$ and MeOH with increasing polarity. The fractions were monitored by TLC (detection under UV and with 5% alcoholic ferric chloride spray). Fraction containing bitter compound was crystallised with acetone. Recrystallisation with MeOH afforded pure white bitter compound **1** (140 mg). The m.p., UV, MS and NMR data were obtained for **1**. Compound **1** (90 mg) was acetylated with $Ac_2O$ (2 ml) and pyridine (2 ml) at room temperature by keeping overnight to yield the pentaacetate **2** as a white powder. The m.p. and $^{13}C$ NMR data were obtained for **2**.

#### 3.3.1. 2′-(2,3-Dihydroxybenzoyloxy)-7-ketologanin (1)

Crystals, UV $\lambda_{max}$ nm = 218, 324; $R_f$ value – 0.40. M.p. 202–203 °C; SIMS *m/z* 525 $[M+H]^+$. EIMS *m/z* (rel. int.) 524.47 (10) {M]+, 474 (5), 298 (40), 226 (100), 198 (85), 167 (70), 137 (90), 107 (15), 85 (55). $^1H$ and $^{13}C$ NMR (Table 1).

### *3.3.2. 2′-(2,3-Dihydroxybenzoyloxy)-7-ketologanin pentacetate* (**2**)

White amorphous solid, $R_f$ value – 0.60; M.p. 171–172 °C; $^{13}C$ NMR (100 MHz. $CDCl_3$): δ 217.4 (C-7), 170.5 (C-7″), 166.0 (C-11), 150.1 (C-3), 143.9 (C-2″), 143.6 (C-3″), 128.4 (C-4″), 128.2 (C-6″), 126.0 (C-5″), 123.6 (C-1″), 110.7 (C-7), 96.1 (C-1′), 93.3 (C-1), 72.5 (C-5′), 72.0 (C-2′), 70.9 (C-3′), 68.4 (C-4′), 61.7 (C-6′), 51.1 (OMe at C-11), 45.0 (C-9), 43.0 (C-8), 42.1 (C-6), 26.3 (C-5), 13.1 (C-10), 161.8–170.5 (5 × acetate C=O), 20.4–20.6 (5 × acetate Me).

Acknowledgements: The facilities provided by the University of Horticulture and Forestry, Nauni Solan, India, for undertaking the studies are greatly acknowledged.

## References

1 Nadkarni, K. M.; Nadkarni, A. K.: Indian Materia Medica, Vol. II. p. 309, Popular Prakashan, Bombay, 1976

2 The Indian Pharmaceutical Codex, Vol. I. Indigenous Drugs. p. 64–65, Council of Scientific and Industrial Research, New Delhi, India 1953

3 Jensen, S. R.; Schripsema, J.: Chemotaxonomy and pharmacology of Gentianaceae. In: Struwe, L.; Albert, V. (eds.): Gentianaceae-Systematics and Natural History, p. 573, Chembridge University Press, 2002

4 Gross, G. A.; Sticher, O.; Anklin, C.: Helv. Chim. Acta **69**, 156 (1986)

5 Chulia, A. J.; Kaouadji. M.: J. Nat. Prod. **48**, 54 (1985)

6 Mpondo, E. M.; Garcia, J.: Phytochemistry **29**, 643 (1990)

7 ISI Web of Science database, Institute of Scientific Information, MIMAS, Manchester Computing, University of Manchester (2003). Available online at: http://wos.mimas.ac.uk/

8 Bergeron, C.; Marston, A.; Gauthier, R.; Hostettmann, K.: Phytochemistry **44**, 633 (1997)

9 Muller, A. A.; Kufer, J. K.; Dietl, K. G.; Reiter, S. A.; Grau, J.; Weigend, M.: Phytochemistry **52**, 67 (1999)