Isolation of Terpenoids from *Cassia siamea*

Deepa Chauhan

Department of Chemistry,

M.S.College,Saharanpur

INTRODUCTION

The genus Cassia is represented abundantly in North India. In traditional medicine, extracts of these plants were reported to exhibit medicinal properties (1-4). As a part of our continuing studies on terpenoids from genus Cassia we examined C. siamea. Stem bark of this plant is used as mild pleasant & safe purgative. C.siamea leaves can be used as manure. A decoction is given in diabetes & a paste can be used as dressing of ring worm & chilblains. From this mixture, we report here the isolation & characterization of two terpenoids viz. Oleanolic acid 3-6'-O-methyl-β-D- glucoronoranoside (1). Oleanolic acid 3-O-α-L-rhamnopyranosyl (1→3)-6-O-methyl-β-D-glucoronopyranoside (2).

RESULTS AND DISCUSSION

Spectral analysis of 1 indicated that it was a mono-glycoside of an oleanane –type triterpenoid acid. Hydrolysis by mineral acid afforded glucuronic acid. The configuration of sugar linkage was determined as β from the ^{1}H NMR signal pattern of this anomeric portion (J=8 Hz). the 13 C NMR spectrum (Table 1) showed that the δ value of the signals, due to the aglycone moiety, closely resembled the corresponding signal in oleanolic acid (5) except those attributable to the deshielded C-3 (+ 10.9 ppm) and the shielded C-2 (-1.6ppm),thus indicating glycosylation at the C-3 position. The sugar carbon data (Table-2) prove the pyranose form of the sugar moiety and revealed a noticeable difference in the δ value of C-6' (-2 ppm), in comparision to the corresponding value in related compounds with a free glucuronic acid moiety (6).

Table 1. 13C NMR data of the aglycone moieties of terpenoids 1-2 in pyridine d5 (ppm)

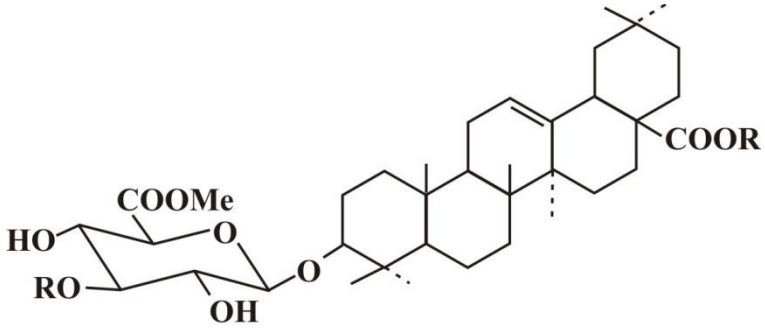
C	1	2
1	38.7	38.6
2	26.5	26.6
3	89.1	89.4
4	39.5	39.5
5	55.8	55.8
6	18.5	18.4
7	33.2	33.2
8	39.7	39.8
9	48.0	48.0
10	37.0	37.0
11	23.8	23.8
12	122.6	122.5
13	144.9	144.9
14	42.0	42.0
15	28.3	28.4

This Observation, together with the presence of two signals at δ 52.0, assigned to a carbomethoxyl group (3.7 ppm in 1H NMR), and at δ 180.1, due to a free carboxylic function at C-17 of the aglycone, suggested the structure of oleanolic acid 3-O-6' –O- methyl-β-D-glucuronopyranoside for 1. The FAB mass spectrum was in agreement with the proposed structure and exhibited [M]+ at m/z 669 [C37 H58 O9+ Na]. Two saponins with related structures have already been related from Schefflera impressa (Araliaceae), these are hedegagenin 3-O-β-D-glucuronopyranoside -6'-O- methyl ester and its 23-hydroxy ursolic acid analogue [7].

Acid hydrolysis and ^{13}C NMR analysis of 2 suggested the presence of 6'-O-methyl-D-glucuronopyranosyl and L-rhamnopyranosyl moieties. The configurations of their linkages were determined as β-and α- respectively, from the ^{1}H NMR coupling constants (J=8 and 2 Hz). The ^{13}C NMR data (Table 1) showed signals in agreement with oleanolic acid substituted at the C-3 position by a sugar chain.

The signals due to the sugar moieties (Table 2) demonstrated by aglycosylation shift of C-3' (+4.2ppm) and C-4' (-0.3ppm) signals in the 6'-O-methyl-β-D-glucuronopyranosyl moiety due to substitution at its C-3' position by terminal α-L- rhamnopyranosyl moiety (6). The FAB mass spectrum was consistent with the above observations displayed $[M]^{1}$ at m/z 815

[$C_{43}H_{68}O_{13}$+Na]. Thus compound 2 was assigned the structure of oleanolic acid 3-O-α-L-rhamnopyranosyl (1→3)-6'-O-methyl-β-D-glucuronopyranoside.



	R	R'
1	H	H
2	α-L-rha	H

Table 2. ^{13}C NMR data of the sugar moieties of terpenoids 1-2 in pyridine-d,(ppm)

C	1	2
Glc-UA-Me		
1	107.3	107.0
2	75.4	75.7
3	78.0	82.2
4	73.1	72.8*
5	77.2	77.1
6	107.8	17.06
OMe	52.0	52.1
Rha		
1		102.9
2		71.4
3		72.5*
4		74.1
5		69.8
6		18.9

GIc= glucose Glc.UA- Me= glucuronic acid methyl ester. Rha = Rhamnose *Signals may be interchangeable

- General instrumental analysis was carried out as described in ref. [8].

- *Extraction and isolation. Cassia simea* was collected from Allahabad, U.P. India and identified by Botanical survey of India Central Circle Allahabad, where a voucher specimen is deposited. Dried aerial parts (3.kg) were soaked in 60% aq. EtOH. The residue of the extract (75 g) was dissolved in MeOH and after dilution with Me_2CO a ppt of crude terpenoid (20 g) was collected. Silica gel CC ($CHCI_3$-MeOH 90:10) afforded several fractions from which crude terpenoids 1-2 were isolated after repeated prep. TLC ($CHCl_3$-MeOH-EtOAC-H_2O. 28:30:35:5). Purification of the saponin was carried out by HPLC on a Develosil ODS-10 column (20mm x 25 cm) using CH_3CN-H_2O (55:45) + 0.05% TFA as mobile phase at flow rate of 6.5 ml min-1. The content of the terpenoids 1-2 in the plant material was estimated as 0.02 & 0.03.

- Oleanolic acid 3-0-6'-O-methyl-(3-D-glucuronopyranoside (1). Amorphous powder. $[\alpha]_D^{22}$ -18.56 ^{1}HNMR (500 MHz, pyridine-d_5):δ 0.82, 0.96 0.97, 0.99, 1.02, 1.31 (s, 7 × Me), 3,29 (dd, J=10.2 Hz, H-18), 3.38 (dd, J = 12.4 Hz, H-3). 3.73 (s. CO_2Me), 4.07 (t, J = 8.5 Hz, Glc. UA H-2). 4.25 (t, J = 9 Hz. Glc. UA H-3). 4.46 (t, J =9 Hz, Glc.UA H-4), 4.58 (d, J=9.5 Hz, Glc. UA H-5), 4.98 (d, J = 8 Hz, Glc.UA H-1), 5.48 (t, J = 2.5 Hz, H-12). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

- Oleanolic acid 3-O- α-L-rhamnopyranosyl (1→3)-6-O-methyl-β-D-glucuronopyranoside (2). Amorphous powder: ^{1}H NMR (500 MHz. pyridine-d_5,): δ0.81, 0.92, 0.96, 0.98, 1.02, 1.25, 1.31 (s. 7 × Me), 1.69 (d, J = 6 Hz. Rha H-6), 3.29 (dd, J = 10, 3 Hz, H-18), 3.31 (dd, J = 11, 4Hz, H-3), 3.77 (s. CO_2Me). 4.04 (t, J=8 Hz, Glc.UA H-2), 4.3 (t, J = 9.5 Hz, Glc. UA H-3), 4.39 (t. overlapped, Glc.UA H-4), 4.53 (d, J = 8.5 Hz, Glc. UA H-5), 4.73 (d. J = 2 Hz. Rha H-1), 4.88 (d, J = 8 Hz, Glc.UA H-1). 5.47 (t, J = 2 Hz, H-12). ^{13}C NMR (pyridine-d_5): see Tables 1 and 2.

- Acid hydrolysis. Saponin (2 mg) was heated under reflux with 5 ml of 2 N HCI-MeOH (1:1) for 2 hr. After usual work-up, the solution was checked on PC for detection of sugar components.

REFERENCES

1. Krfitikar K R, Basu B P, Indian Medicinal Plant 1975, p. 854.
2. Poiter & Das BC, Bai A. Janot M., Pounrrat A, Pourrat H, Bull. Soc. Chim Fr. 1966; 3458-3465.
3. Srivastava M, Singh J, Int. J. Pharmacognosy, 1994, 32-199-200
4. Chopra, R.N., Nayar, S.L. and Chopra I.C. (1956) *Glossary of Indian Medicinal Plants,* p.116. Council of Scientific & Industrial Research, New Delhi.
5. Kizu, H. and Tomimori, T. (1982) *Chem. Pharm. Bull.* 30, 3340.
6. Borel, C., Gupta, M. P. and Hostettmann, K. (1987) *Phytochemistry26,* 2685.
7. Srivastava, S.K. and Jain, D.C. (1989) *Phytochemistry28,* 644.
8. Miyase. T., Kohsaka H. and Ueno. A. (1992) *Phytochemistry* 31, 2087.