19-Oxo-5-α-Carda-14,20(22)-Dienolide-3-O-β-D-Glucopyranoside from *Millettia ovalifolia* Roots

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The roots of *Millettia ovalifolia*, commonly known as Gauj belongs to n.o. Leguminosae. The roots of this plant are reported to be poisonous. The ethyl acetate soluble fraction of the concentrated ethyl alcohol soluble part of its roots when subjected to column chromatography yielded a light yellow coloured compound, m.f. $C_{29}H_{40}O_9$, m.p. 263–64°C, $[\alpha]_D^{18} = 36.7°$ (CH_3OH), and $M^+ = 370$, which on various chemical degradations, colour reactions and spectral analysis was identified as 19-oxo-5-α-carda-14,20 (22)-dienolide-3-O-β-D-glucopyranoside [compound **C-1**]..

INTRODUCTION

The plant *Millettia ovalifolia*[1, 2] is commonly known as Gauj in Hindi and belongs to the natural order Leguminosae. It is cultivated in the outer Himalay in Sikkim. It is also found in the forest of Dehradun. The roots of this plant are reported to be poisonous. In view of its poisonous nature, the plant has been under phytochemical investigations by ealier workers who have already reported the presence of important biologically active compounds in it[3–7].

Its roots were therefore subjected to further plytochemical investigations and the ethyl acetate soluble part of the concentrated alcoholic extract of roots when worked up by column chromatography yielded a novel cardenolide which was identified by chemical degradations, colour reactions and spectroscopic studies as 19-oxo-5-α-carda-14,20(22)-dienolide-3-O-β-D-glucopyranoside **(1)**.

EXPERIMENTAL

The roots of the plant *Millettia ovalifolia* were collected personally with the help of forest department from Dehradun (India) and herbarium specimen has been preserved also.

Extraction of the Roots: About 5 kg of chipped roots were completely dried and powdered and then defatted with petroleum ether in a soxhlet extractor till completely defatted. The defatted powdered roots were then taken out and extracted with rectified spirit in a 5 litre round bottomed flask for several days and the extract was filtered while hot and concentrated to get a brown viscous mass. This brown viscous mass was subsequently subjected to extraction with various solvents of increasing polarity. The ethyl acetate soluble part responded

to positive Keller Kiliani[8] test and Legal[9] tests, thereby showing the presence of cardenolide in it.

On TLC examination (solvent system n-butanol : acetic acid : water) on silica gel plates (spraying reagent Kedde's[10] reagent 2% 3,5-dinitrobenzoic acid, in alkaline methanol) it showed two spots. This fraction was therefore subjected to column chromatography over silica gel and eluated with chloroform : ethylacetate : methanol (3 : 3 : 4) and the eluates which showed single spot of same R_f value were mixed and solvent removed under reduced pressure followed by crystallisation from acetone to get a light yellow powdered compound, m.f. $C_{29}H_{40}O_9$, m.p. 233–235°C, $[\alpha]_D^{18} = 36.7°$ (MeOH), UV λ_{max} 220, 274, IR λ_{max} 3450, 2985, 2855, 1745, 1720, 1650, 1445, 1030, 880 and 690, and $M^+ = 552$, 369, 353, 352, 341, 332, 287, 269, 247, 229, 217, 176, 161 and 111. Found (%) C = 65.80, H = 7.15; calculated (%) for $C_{29}H_{40}O_9$, C = 65.41, H = 7.52.

Acetylation of C-1: 50 mg of C-1 was suspended in 10 mL of pyridine and 10 mL of acetic anhydride and stirred at room temperature for overnight. The solution was evaporated *in vacuo* and the residue was crystallised from ethyl acetate to get very light yellow coloured compound, m.p. 270–71°C, m.f. $C_{37}H_{48}O_{13}$ $M^+ = 695$, ^1H NMR ($CDCl_3$ 100 MHz), $\delta = 0.8$, 1.25–2.00, 2.04, 2.08, 2.09, 2.12, 2.27, 3.35–4.20, 4.35, 4.50, 5.00, 5.48, 5.88 and 10.2. Found(%) C = 64.02, H = 7.00; calculated(%) C = 63.88, H = 6.90.

Acid Hydrolysis of C-1: The compound C-1 was hydrolysed with 7% ethanolic H_2SO_4 by refluxing for about 10 h. Thereafter the ethyl alcohol was distilled off when aglycone C-2 was obtained. The hydrolysate was neutralised with $BaCO_3$ and $BaSO_4$ was filtered off and the filtrate was concentrated under reduced pressure. On paper chromatographic examination the sugar was identified as glucose by Co-PC and Co-TLC. The sugar on quantitative estimation showed the presence of one molecule of glucose.

Identification of the aglycone C-2: The aglycone was obtained as white crystalline solid m.p. 233–234°C, m.f. $C_{23}H_{30}O_4$. Found(%) C = 75.00, H = 8.24; calculated (%) for $C_{23}H_{30}O_4$ C = 74.56, and H = 8.16; $[\alpha]_D = -38.9°$ (CH_3OH), UV λ_{max} 220 and 270, IR 3470, 2980, 2865, 2835, 1750, 1720, 1650, 1450, 1375, 1340, 1320, 1180, 1150, 1030 and 890 cm^{-1}.

Acetylation of C-2 to C-3: 60 mg of suspension of C-2 in pyridine (10 mL) and acetic anhydride (10 mg) was kept overnight at room temperature. The solvent from this mixture was removed by evaporation and the residue was crystallised from CH_3OH to get colourless needles, m.p. 249–250°C. The acetyl derivative analysed for m.f. $C_{25}H_{32}O_5$. Found (%) C = 72.75, H = 7.80; calculated (%) for $C_{25}H_{32}O_5$, C = 72.79, H = 7.81. It showed signals in ^1H NMR spectrum of $\delta = 0.82$ (S, 3H—CH_3) 1.25 to 2.00 (Polymethylene —CH_2— and —CH=), 2.05 (5, 3H, C_3—OAc), 2.76 (d, 1H, J = 4.8 Hz, H), 4.60 (t, 1H, J = 3.5 Hz, C_3—H), 5.00 (ddd, 2H, J = 2.1 Hz, C-21H), 5.46 (m, 1H, C-15H), 5.88 (S, 1H, C-22H), 10.02 (S, 1H, —CHO) and $M^+ = 412$.

Methylation of C-2 to C-4: 30 mg of C-2 was taken in acetone (10 mL) along

with K_2CO_3 (100 mg) and $(CH_3)_2SO_4$ (1 mg). This reaction mixture was kept for 5 h at 60°C, and thereafter diluted with water and extracted with diethyl ether. Removal of diethyl ether and subsequent crystallisation of the product from methanol gave C-4 as white coloured amorphous compound, m.p. 191–192°C, m.f. $C_{24}H_{32}O_4$. Found (%) C = 74.90, H = 8.40; calculated (%) for $C_{24}H_{32}O_4$, C = 74.97, H = 8.4.

Jones[11] *Oxidation of C-2 to C-5:* 50 mg of C-2 was taken with 40 mL of diethyl ether to which 100 mg of $K_2Cr_2O_7$ was added along with 1 mL of concentrated H_2SO_4 and 5 mL of H_2O. The reaction mixture was shaken well and allowed to stand overnight. The residue was crystallised from ethyl-acetate : hexane 1 : 1 to get C-5; m.p. 241–242°C, EIMS $M^+ = 368$, m.f. $C_{23}H_{28}O_4$ Found (%) C = 75.00, H = 7.60; calculated (%) for $C_{23}H_{28}O_4$, C = 74.98, and H = 7.66.

Catalytic Hydrogenation of C-2 to C-6: 30 mg of C-2 was taken in 25 mL of ethyl alcohol and hydrogenated in presence of PtO_2 with H_2 at room temperature for about 4 h. The solvent was removed under reduced pressure and the residue crystallised from CH_3OH, m.f. $C_{23}H_{34}O_4$, EIMS $M^+ = 374$, m.p. 170–171°C, found (%) C = 74.0, H = 9.2 calculated (%) for $C_{23}H_{34}O_4$, C = 73.8, H = 9.15.

RESULTS AND DISCUSSION

The compound C-1 ($C_{29}H_{40}O_9$) was obtained as light yellow coloured substance. It responded to positive Kedde's, Legal and Keller Kiliani test, thereby confirming its cardenolide nature. Peak in the IR spectrum at 3300 cm^{-1} indicates the presence of free OH; so it is the compound C-1.

On acid hydrolysis with 6% ethanolic H_2SO_4 the compound C-1 gave a cardiogenin C-2, m.f. $C_{23}H_{30}O_4$, m.p. 233–235°C, $M^+ = 370$ and sugar identified as glucose.

Its IR spectrum displayed peak at 3450 cm^{-1} confirming the presence of free OH group. The cardenolide underwent acetylation using acetic anhydride and pyridine $C_{25}H_{32}O_5$, $M^+ = 412$, and m.p. = 229–30°C. The cardenolide was subjected to Jones[11] oxidation when it gave a compound, m.p. $C_{23}H_{28}O_4$, $M^+ = 368$, which responded to positive Zimmermann test[12] for >C=O group.

The cardenolide analysed for m.f. $C_{23}H_{30}O_4$, m.p. 233–35°C, m/e = 370. Its cardenolide nature was supported by the fragmentation pattern due to loss of M-18 (m/z = 352), and subsequently followed by M-29 (m/z = 341). The mass spectrum showed characteristic peak at m/z = 111, which is specific for the cardenolide.

Its UV spectrum showed λ_{max} at 270 nm, which is characteristic of conjugated carbonyl group, and the presence of a lactone ring was confirmed by a peak at the IR spectrum in 1745 cm^{-1}. Its ^1H NMR spectrum displayed a broad singlet at $\delta = 5.88$ (1H) and a triple bond of $\delta = 5.07$ (2H) which is characteristic of a lactone ring. The presence of lactone ring at C-17 was confirmed by the fact that the O-methyl ether derivative of the cardenolide on $KMnO_4$ oxidation gave

another compound, m.f. $C_2H_{30}O_4$, which was found to be identical to 3β-methoxy-19-oxo-5α-di-14-enoic acid.

The IR spectrum band at 1650 cm^{-1} indicated the presence of unsaturation. As such the compound was subjected to catalytic hydrogenation when it formed a tetrahydro derivative of the cardenolide $C_{23}H_{34}O_4$, m.p. 168–70°C, $M^+ = 374$, thereby establishing the presence of two double bonds in it.

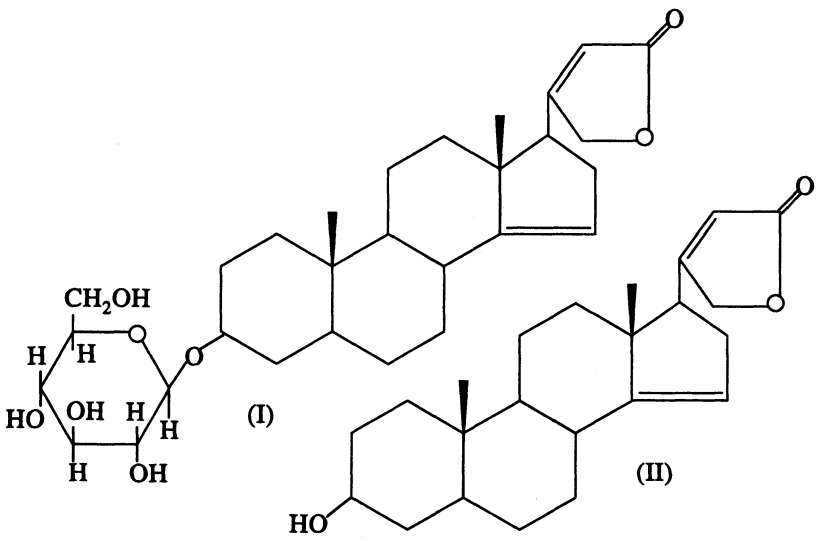
The cardenolide gave deep red colour in pyridine with alkaline solution of sodium nitroprusside which is characteristic of position of double bond at C-20 (22)[13], which was further established by λ_{max} at 218 in the UV spectrum of the compound.

The presence of olefinic bond in the molecule of the cardenolide was established by positive TNM test[14] and its position was fixed at C-14 in the cardenolide.

The above evidences on compilation concluded that the cardenolide C-1 is 19-oxo-5-α-carda-14,20(22)dienolide-3-ol (II).

Position of attachment of sugar to C-2 in C-1: The position of attachment of sugar residue to cardiogenin was fixed at C-3, because the aglycone C-2 (cardiogenin) responded to positive Zimmermann test, while the glycoside C-1 did not respond to this test, thus confirming that the sugar was attached to C_3—OH group in the cardenolide glycoside. The point of attachment of the aglycone to sugar was confirmed by methylation of the glycoside followed by hydrolysis when the sugar was obtained, which was identified as 2-3-4-6-tetra-O-methyl-D-glucose thereby confirming that C-1 of the sugar was involved is glycosilation.

Attachment of sugar to aglycone C-2: The compound C-1 was treated with $(CH_3)_2SO_4$ in aq. NaOH, thereby giving methylated glycoside. The methylated



glycoside was hydrolysed with 4-N H_2SO_4. The hydrolysate when worked up by the usual method identified the sugar as 2-3-4-6-tetra-O-methyl-D-glucose.

Periodate oxidation: 40 mg of the compound C-1 was dissolved in CH_3OH and treated with $NaIO_4$ for 50 h. The amounts of formic acid liberated and periodate consumed were estimated by the method of Jones.

Nature of the glycoside linkage in C-1: The cardenolide glycoside C-1 was treated with enzyme emulsion at room temperature for about 30 h when the glycoside C-1 underwent hydrolysis to give glucose thereby confirming that the glucose was linked to aglycone *via* β-linkage.

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(*Received*: 4 December 1999; *Accepted*: 11 January 2000) AJC-1977