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A Novel Anthraquinone Glycoside From Impatiens Balsamina Linn. Seeds

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The present paper describes the isolation and identification of a novel anthraquinone glycoside 1,3,dimethoxy-6, methyl anthraquinone-8-O-β-D-glucopyranosyl (1 → 4)-O-α-L-rhamnopyranoside.

INTRODUCTION

Medicinal use attributed to members of the natural order *Balsaminaceae* are well known and so it attracted our attention on *Impatiens balsamina* Linn[1,2] (N. O. *Balsaminaceae*) is known Gulmendi in Hindi and is distributed throughout in India. Alcoholic extract of the flowers is reputed for curing pains in the joints. When taken internally it acts as an emetic cathartic diuretic. The flowers are cooling and tonic useful when applied to burns and scalds.

Earlier workers[3,4] have already reported the presence of 2-hydroxy-1,4-naphthaquinone, 2-methoxy-1,4-naphthaquinone from the seeds and leaves of the plant. The present paper deals with the isolation and identification of a novel anthraquinone glycoside 1,3, dimethoxy-6, methyl anthraquinone-8-O-β-D- glucopyranosyl (1→4)-O-α-L-rhamnopyranoside.

RESULTS AND DISCUSSION

The water soluble part of the rectified spirit extract of the 95% concentrated ethanolic extract of dried and powdered seeds gave crude glycoside (0.66%), which was found to be homogeneous on TLC (benzene) and on column chromatography yielded orange needles from (ethylacetate : light petroleum ether 1 : 1), m.pt. 238–40°C, molecular formula $C_{29}H_{34}O_{14}$[1] $M^+ = 606$ ($R_f = 0.64$). It gave positive Molisch test and responded to all the colour tests[5–8] charaacteristic of an anthraquinone glycoside.

On acid hydrolysis with 7% ethanolic H_2SO_4 [1] gave an aglycone [2] (0.42%) m.pt. 212°, $C_{17}H_{14}O_5$ and sugar moieties. The sugars were identified as rhamnose and glucose (by CO-PC and CO-TLC). The anthraquinone skeleton was further supported by the isolation of 2-methyl anthracene (m. pt. 205–206°) from Zn-dust distillation of [2]. ^1H NMR spectrum of monoacetyl derivative of [2] showed signals at $\delta = 7.18$ (d, J = 2.4 Hz, $C_2 - 1H$), $\delta = 7.38$ (d, J = 2.5 Hz, $C_4 - 1H$), $\delta = 7.92$ (d, J = 2.5 Hz, $C_5 - 1H$), $\delta = 6.98$ (d, J = 2.0 Hz, $C_7 - 1H$), $\delta = 3.98$ (s, 3H, $C_1 - OCH_3$), $\delta = 3.86$ (s, 3H, $C_3 - OCH_3$), $\delta = 2.53$ (s, 3H, C_6

$-CH_3$), $\delta = 2.56$ (s, 3H, C_8 – OA)C; UV λ_{max} (EtOH) 265, 282 and 414 nm; EIMS M^+ 298, m/z = 270, 242, 214, 213, 136, 106; IR ν_{max}^{KBr} 3300, 2947, 2892, 1632, 1680, 1666, 1656, 1598, 1478 cm^{-1}.

The IR peak at ν_{max}^{KBr} 3300 cm^{-1} indicated the presence of free OH group. Preparation of a mono-acetyl derivative $C_{19}H_{16}O_6$; m. pt. 188° M^+ 340, suggested one acetylable OH group in the [2]. The [2] gave a red complex with zirconium nitrate solution, soluble in HCl showing the presence of a hydroxyl group[9] at position C–8.

The ethanolic of the [2] formed a complex with ethanolic copper sulphate showing the presence of a hydroxyl function in the α-position of the >C = O group[10].

The presence of only α-hydroxyl group is further supported by the peaks at 1632 cm^{-1} and 1680 cm^{-1} in the IR spectrum and λ_{max} at 414 nm in the UV spectrum of the [2].

A peak in the IR spectrum of [2] at 2892 cm^{-1} indicated the presence of $-OCH_3$ group(s) in it. Methoxy group estimation (21.12%) was done by Zeisel[11] method which confirmed the presence of two methoxyl groups in [2].

The [2] gave red colour on treatment with concentrated H_2SO_4 showing the presence of at least one methoxyl group in any α-position[12]. Thus of the two methoxyl groups present in the [2], one is at β-position and the other is at α-position.

The ^1H NMR of acetylated derivative of the [2] showed singlet at $\delta = 3.98$ and $\delta = 3.86$ integrating for 3 protons and confirmed the presence of two methoxyl groups at positions 1 and 3 in the ring (A).

The *m*-di-substituted pattern in the ring A and C is supported by the ^{1}HNMR spectrum of acetylated derivative of the [2] showed signal at $\delta = 7.18$ (d, J = 2.4 Hz, C_2—1H), $\delta = 7.38$ (d, J = 2·5 Hz, C_4—1H). $\delta = 7.92$ (d, J = 2.5 Hz, C_5—1H) $\delta = 6.98$ (d, J = 2.0 Hz, C_7—1H).

In the IR spectrum peak at ν_{max}^{KBr} 1478 cm^{-1} indicated the presence of methyl group in the [2]. Estimation of methyl group by the semi-micro apparatus as mentioned by Belcher and Godbert[13] (4.9992%) confirmed the presence of one methyl group in the [2] and [2] was identified as 1, 3, dimethoxy-8-hydroxy-6-methyl anthraquinone.

The [2] gave a red complex with zirconium nitrate solution, soluble in HCl, indicating the presence of —OH group at position C–8 in the [2] but its absence in the [1], clearly indicating the involvement of 8—OH in glycosylation with the diasaccharide moiety. The [1] on graded hydrolysis by Killani mixture liberated first glucose followed by rhamnose indicating that glucose was the terminal unit and rhamnose was attached to aglycone. Periodate oxidation[14] of the [1] indicated the presence of a disaccharide having both the units in pyranose form and on enzymatic[15] hydrolysis with emulsion gave glucose and unhydrolysed agly-rhamnose,

which on further hydrolysis with enzyme tokadiastase yielded aglycone[2] and rhamnose proving β-linkage between glucose and rhamnose and α-linkage between rhamnose and aglycone[2].

Acid hydrolysis of permethylated glycoside resulted 2, 3, 4, 6-tetra-O-methyl glucose and 2 : 3 di-O-methyl L-rhamnose thereby confirming that glucose was attached to rhamnose by (1 → 4) linkage and rhamnose with aglycone [2] by C_1. Thus [1] was identified as; 1,3, dimethoxy-6, methyl anthraquinone-8-O-β-D-glucopyranosyl (1 - → 4)-O-α-L-rhamnopyranoside and was further supported by ^{1}HNMR spectrum of its hexa-acetyl derivative showed singlet at δ = 7.16 (d, J = 2.4 Hz, C_2—1H), δ = 7.35 (d, J = 2.5 Hz, C_4—1H), δ = 7.94 (d, J = 2·5 Hz, C_5—1H), δ = 6·96 (d, J = 2.0 Hz, C_7—1H), δ = 3·96 (s, 3H, C_1—OCH_3), δ= 3.84 (s, 3H, C_3—OCH_3), δ = 2·51 (s, 3H, C_6—CH_3), δ = 4.38 (d, J = 7.5 Hz, 1-anomericproton—$C_{1''}$), δ = 4.26 (d, J = 2 Hz, 1-anomeric proton—$C_{1'}$, δ = 2.08 (s, 3H, C_2^*—OAc), δ = 3.00 (s, 3H, $C_{3'}$—OAc), δ = 0·74, (s, 3H, $C_{6''}$—CH_3), δ = 3.02 (s, 3H, $C_{2''}$,—OAc), δ = 2.98 (s, 3H, $C_{3''}$,—OAc), δ = 2.04 (s, 3H, $C_{3''}$,—OAc), δ = 3.90 (s, 3H, $C_{6''}$,—OAc), δ = 4.61–4.82 (m, 4-protons of rhamnosyl unit), δ = 5.44 (m, 6-protons of glucose unit).

EXPERIMENTAL

Dried and powdered seeds were extracted exhaustively with 95% ethanol. The extract was concentrated under reduced pressure and the concentrated extract poured into water. The concentrated water soluble part was successively extracted with rectified spirit and concentrated under reduced pressure. The concentrated extract poured in 400 ml of water and the coloured precipitate (8·6 gm) thus obtained was subjected to column chromatography.

The coloured precipitate showed a single spot on tlc Si, gel G plates using benzene. The fraction was purified over a Si gel column. (60-120 mesh) and eluted with ethylacetate : light petroleum ether (1 : 1) providing compound [1] : orange coloured needles (0.66%), m.pt. 238-240° (Found : C = 57, 38%, H = 5.58%, $C_{29}H_{34}O_{14}$ calcd. for : C = 57.42% H = 5·61%): UVλ_{max} (EtOH) 262, 292 and 404 nm; EIMS M^+ 606, m/z = 443, 427, 298, 270, 242, 214, 213, 136, 106; IR ν_{max}^{KBr} 3296, 2932, 2896, 1760, 1690, 1678, 1660, 1628, 1600 and 1470 cm^{-1}.

Acid hydrolysis of the glycoside

400 Mg of compound was refluxed with 7% alc. H_2SO_4 (50 ml) in a 150 ml R.B. flask on a water bath for 8 hrs. Then (50 ml) of water was added to the reaction mixture and alcohol was removed by its distillation under reduced pressure, when it yielded on aglycone [2] as a

precipitate which was separated. The aqueous layer was worked up separately for the identification of sugars and on paper chromatographic examination (BAW 4 : 1 : 5) showed the presence of D-glucose and L-rhamnose.

Zn-Dust distillation of the aglycone

The aglycone (40 mg) and Zn-dust (2 gm.) was taken in a pyrex glass tube sealed and heated upto dull red whereupon a yellow coloured compound m.pt. 205-206°C was obtained at the upper end of the tube and was identified as 2-methyl anthracene (m. pt. 205-206°C).

Acetylation of the Aglycone

50 Mg of the compound was dissolved in 2 ml of pyridine and treated with 1 ml. of acetic anhydride. The mixture was heated for 10 minutes over the water bath, left overnight, precipitated with ice water, washed and crystallised acetyl derivative obtained m.pt. 188°, $C_{19}H_{16}O_6$, M^+ 340.

Graded hydrolysis of Compd. [1]

Compd. [1] was mixed with killani mixture and left for two days partial hydrolysis was monitered by pc. After appearence of glucose the unhydrolysed agly-rhamnose was separated m.pt. 202°, analysed for $C_{23}H_{24}O_9$.

Periodate Oxidation

Compd. [1] was dissolved in methanol and kept with sodium meta-periodate for 2 days, liberated formic acid and consumed periodate was estimated by Jone's method.

Parmethylation and Acid hydrolysis

Compd. [1] was treated with CH_3I and Ag_2O in dimethyl formamide at room temp. After two days the reaction mixture was filtered and the residue was washed with chloroform. The filtrate was conc. and hydrolysed by 22% alc. H_2SO_4. After usual work up methylated sugars were identified on PC as; 2, 3, 4, tri-O-methyl-L-rhamnose and 2, 3, 4, 6-tetra-O-methyl-D-glucose.

Enzymatic hydrolysis

Compd. [1] dissolved in ethyl alcohol and an aqueous solution of emulsin was mixed and left at room temp. for 48 hrs. The examination of hydrolysate showed presence of glucose. The unhydrolysed agly-rhamnose was separated and again treated with aqueous soln. of tokadiastase for 8 hrs resulting into aglycone [2] and rhamnose.

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