2-Desacetyl-8-Epi-Xanthumanol-4-O-β-D-Galactopyranoside: The Potential Antitumour Sesquiterpenoidal Lactone from *Xanthium spinosum* Bark

A.H. ANSARI and K.S. DUBEY*
Department of Chemistry
Govetrnment P.G. College (R.D. University)
Mandla-481 661, India

Xanthium spinosum Linn is reported to be used against hydrophobia, rabies and intermittent fever. It has diuretic properties and is reported to cure diarrhoea and cancer. The ethylacetate-soluble fraction of the concentrated alcoholic extract of *Xanthium spinosum*, bark when worked up chromatographically, yielded a colourless amorphous powder, m.f. $C_{21}H_{32}O_9$, $M^+ = 428$ and m.p. 157–158°C. Various colour reactions, chemical degradations and spectroscopic studies identified it as 2-desacetyl-8-epr-xanthumanol-4-O-β-D-galactopyranoside (1).

INTRODUCTION

Xanthium spinosum Linn is reported to be used against hydrophobia, rabies and intermittent fever. In this work we extracted a potential antitumour sesquiterpenoidal lactone, *i.e.*, 2-desacetyl-8-epr-xanthumanol-4-O-β-D-galactopyranoside (1).

RESULTS AND DISCUSSION

The bark of *Xanthium spinosum*[1] was chipped, dried, defatted and then extracted with alcohol. The alcoholic extract was filtered while hot and concentrated under reduced pressure to get a brown viscous mass which was then extracted with ethyl acetate and this extract responded to positive tests for xanthanolide and also gave positive Molish test. It was therefore concentrated to a viscous mass and subjected to column chromatography (silica gel-ethylacetate : chlorofrom : benzene 4 : 3 : 3 as eluents) and the eluents having same R_f values were combined and solvent removed to get colourless amorphous powder which was dissolved in methanol and again obtained in amorphous form (D-1).

D-1, analysed for molecular formula $C_{21}H_{32}O_9$, $M^+ = 428$ and m.p. 157–158°C.

Peak at 3500 cm^{-1} in the IR spectrum of D-1 indicated the presence of hydroxyl group in it. D-1 was acetylated with acetic anhydride and pyridine to get an acetyl derivative, molecular formula $C_{31}H_{42}O_{14}$, m.p. 155–156°C and M^+ = 638.

Acetyl group estimation (36%) in D-1 indicated the presence of five hydroxyl groups in it.

The xanthanolide glycoside D-1 on hydrolysis with 8% alcoholic H_2SO_4 give an aglycone and sugar moiety(ies) which were separated by filtration. The structure of the xanthanolide glycoside D-1 was established by studying the aglycone and sugar moiety separately.

Study of the Xanthanolide Aglycone (D-2)

The xanthanolide aglycone D-2 was soluble in methanol and crystallised from CH_3OH–$CHCl_3$ (7 : 3). It analysed for molecular formula $C_{15}H_{22}O_4$, M^+ = 266 and m.p. = 150–153°C. It underwent various colous reactions[2] and the hydroxamic acid test confirming its xanthanolide nature. D-2 showed λ_{max} of 210 nm confirming the presence of an α-methylene-γ-lactone ring in it[3]. It displayed peak at 3500 cm^{-1} in its IR spectrum indicating the presence of hydroxyl group it it[4,5]. On acetylation with acetic anhydride and pyridine, it yielded an acetate (D-3) molecular formula $C_{19}H_{26}O_6$, M^+ = 350 and m.p. 186–87°C. Acetyl group estimation (34.60%), by Wiesenberger[6] method also confirmed the presence of two hydroxyl groups in it. This was further established by the methylation of D-2 by dimethyl sulphate to a methylated product D-4, molecular formula $C_{17}H_{26}O_4$, M^+ = 294 and m.p. 154°C, and subsequent estimation of the methoxyl group (22.08%) by Zeisel's[7] method.

On oxidation with chromic acid D-2 gave an oxidation product (D-5), molecular formula $C_{15}H_{18}O_4$, M^+ = 262 and m.p. 169°C. D-5 showed no absorption peak for hydroxyl group in its IR spectrum and gave negative test for aldehydes, thereby establishing the complete oxidation of D-2 to D-5, and thus concluding that both the hydroxyl groups in D-2 were secondary. Downfield signals in the ^1H NMR spectrum of D-3 at δ = 5.32 and δ = 4.82 confirmed that the hydroxyl groups were at C-2 and C-4.[8]

Peaks in the IR spectrum of D-2 at 1665 cm^{-1} and 890 cm^{-1} indicated the presence of unsaturation in D-2. On catalytic hydrogenation[3] with Pd-$BaCO_3$, [3]D-2 yielded a tetrahydro derivative, molecular formula $C_{15}H_{26}O_4$, m.p. 178–179°C and M^+ = 270, which further supported the presence of two double bonds in D-2.

On sodium borohydride reduction, D-2 yielded a compound D-6, molecular formula $C_{15}H_{24}O_4$, M^+ = 268 and m.p. 139°C, which showed that out of two double bonds, only one underwent hydrogenation and so it must be exocyclic methylenic, whereas the other one must be endocyclic.

^1H NMR spectrum of D-2 displayed signals at δ = 6.10 (C-13 H, J = 7 Hz) and δ = 5.46 (C-13′ H, J = 3 Hz), each of one proton intensity and double doublet at δ = 2.71, C-1 H, J = 2, 2.6, 4 Hz for C_1, C_5d (δ = 3.15, 1 H, J = 4, 8, 2 Hz), and C_5β (δ = 3.26, 1H, J = 8, 2, 9 Hz), establishing endocylic double bond in D-2.[9]

Peak in the IR spectrum of D-4 of 1770 cm^{-1} confirmed the presence of α-β-

unsaturated-γ-lactone ring. The position of fusion of the lactone ring with the seven-membered sesquiterpene lactone in D-2 was fixed at C_7–C_8 because of the appearance of multipet at δ = 2.38 C-7 H and doublet at δ = 4.28 C-8 H in the ^{1}H NMR spectrum of D-2.

Peak in the IR spectrum of D-2 at 1150 cm^{-1} indicated the presence of methyl group(s) in it. The splitting pattern of the methyl signal at δ = 1.20 in the ^{1}H NMR spectrum of D-2 for (C-14 (3H), J = 7 Hz) confirmed the secondary nature of the methyl group fixing it at C-10.[10]

Signals in the ^{1}H NMR spectrum of D-6 of δ = 2.70, C-1 H, and doublet of doublet J = 2.0, 2.5 and 3.0 Hz confirmed the attachment of side chain at C-1.

The coupling constant values $J_{6,7}$ and $J_{7,8}$ (3 and 10 Hz) respectively in the ^{1}H NMR spectrum of D-2 established *trans*-configuration between lactone ring and seven-membered ring system, and also confirmed α-configuration of the C-7 proton. The $J_{9,10}$ (5 Hz) on the other hand confirmed β-configuration of the methyl group at C-10.[11-14]

Thus the above deliberations finally concluded that this cardenolide aglycone was 2-desacetyl-8-epi-xanthumanol (II).

Position of attachment of sugar to the cardenolide aglycone (D-2): The cardenolide aglycone has two free-hydroxyl groups while the acetylated glycoside D-1 was found to be penta-acetyl derivative whose ^{1}H NMR spectrum confirmed the presence of acetyl groups at C-2′, C-4′, C-6′, C-7′ and C-2′, thereby concluding that the OH group of C-4 was involved in the glycoside formation

Study of sugar Hydrolysate: The hydrolysate produced by the hydrolysis of the sesquitesrpenoidal lactone gylcoside D-1 was neutralised with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate on concentration was found to be D-galactose by Co-PC and Co-TLC with authentic sample.

Quantitative estimation of the sugar: Quantitative estimation of galactose in D-1 was done by the procedure of Mishra and Rao[15] which confirmed that the cardenolide glycoside was made up of one molecule of cardenolide and one molecule of galactose.

Permethylation of the D-1 by Kuhn's procedure and subsequent hydrolysis yielded 2′,3′,4′,6′-tetra-O-methyl-D-galactose (by Co-PC and Co-TLC), confirming that C-1 of galactose was involved in glycoside formation.

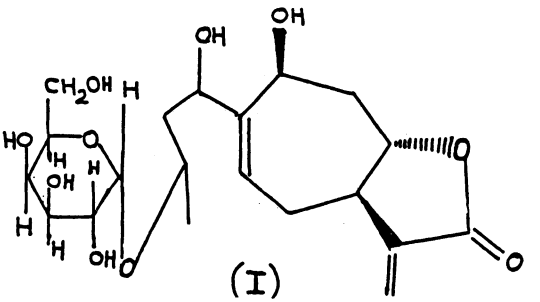
Sodium metaperiodate oxidation of the D-1 showed that the glycoside consisted of one molecule of galactose in pyranose form, whereas enzymatic hydrolysis established its linkage as β.

Thus the cardenolide glycoside D-1 was identified as 2-desacetyl-8-epi-xanthumanol-4-O-β-D-galactopyranoside (I) (Structure on next page).

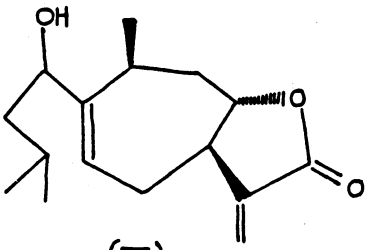
EXPERIMENTAL

Isolation of Cardenolide Glycoside (D-1)

4 kg of dried, chipped and defatted bark of *Xanthium spinosum* was extracted with 5 L of rectified spirit in a 10 L round-bottomed flask under reflux and the extract (3 litre) was concentrated to a brown viscous mass under reduced pressure and extracted with different solvents.



(I)



(II)

The ethyl acetate soluble part (500 mL) was found to respond positive to Molisch test and hydroxamic acid test. It was concentrated to a viscous mass and subjected to column chromatography over silica gel G and eluted with (ethyl acetate : chloroform : benzene 4 : 3 : 3) when eluates from fractions 9 to 15 were found to have the same R_f value 0.32 and then combined and the solvent removed to get a very light yellow-coloured amorphous powder (D-1).

Study of the Cardenolide Glycoside (D-1)

The amorphous mass D-1 responded to characterstic colour reactions of xanthanolides and positive Molisch test. It analysed for molcular formula $C_{21}H_{22}O_9$, $M^+ = 428$ and m.p. 157–158°C.

Acid Hydrolysis of Cardenolide Glycoside (D-1)

500 mg of D-1 was refluxed with 8% alcoholic H_2SO_4 alcoholic (40 mL) in a round-bottomed flask on a water bath for 6 h. 20 mL of water was added to the reaction mixture and alcohol was removed under reduced pressure, when it gave an aglycone D-2 as a precipitate, which was separated by filtration.

The hydrolysate was neutralised with $BaCO_3$ and the precipitated ($BaSO_4$) was filtered off. Concentration of the filtrate under reduced pressure gave a syrupy mass which on examination by paper chromatography (n-butanol : acetic acid : water 4 : 1 : 5) as solvent system and aniline hydrogen phthalate as detecting reagent showed the presence of D-galactose (R_f 0.18).

Study of the Xanthanolide Aglycone (D-2)

D-2 was soluble in ethanol and ethyl acetate and analysed for the molecular formula $C_{15}H_{22}O_4$, $M^+ = 266$, m.p. 150–153°C. It responded positive to hydroxamic acid test and gave colour reactions of xanthanolides.

Acetylation of the Aglycone D-2

The aglycone D-2 (20 mg) was mixed with 10 mL of anhydrous pyridine and 2 mL of acetic anhydride in a round-bottomed flask. The reaction mixture was refluxed for 3 h on a water bath, cooled and poured in cold water (100 mL) to get a precipitate. This precipitate was extracted with solvent ether (20 mL) and the ethereal layer was washed with water and $NaHCO_3$ solution and then dried over anhydrous sodium sulphate and ether removed by evaporation to get acetylated product which crystallized from acetone. D-3 had molecular formula $C_{19}H_{26}O_6$, $M^+ = 350$ and m.p. 186–187°C.

Methylation of the Aglycone (D-2)

100 mg of the aglycone D-2 was mixed with 2 mL of dimethyl sulphate, anhydrous K_2CO_3 and 50 mL of acetone in a round-bottomed flask and refluxed for 4 h. The reaction mixture was cooled and washed with water and acetone was distilled off to get the residue as methylated derivative, molecular formula $C_{17}H_{26}O_4$, $M^+ = 266$ and m.p. 154°C.

Chromium Trioxide Oxidation of D-2

The solution of chromium trioxide (100 mg) in water (20 mL) and concentrated sulphuric acid (0.2 mL) was added to the solution of D-2 in acetone (2 mL) in an ice-bath with constant stirring at room temperature. The whole mixure was then poured into ice-water and extracted with ether to get an oxidation product which analysed for molecular formula $C_{15}H_{18}O_4$, $M^+ = 262$ and m.p. 169°C.

Catalytic Hydrogenation of the Aglycone D-2

60 mg D-2 was taken with 20 mL of methanol and reduced with hydrogen in presence of 5% $Pd/BaCO_3$ to get a tetrahydro derivative, molecular formula $C_{15}H_{26}O_4$, $M^+ = 270$ and m.p. 178–179°C.

Reduction of the Aglycone D-2 with Sodium Borohydride

100 mg of D-2 was mixed with 50 mg $NaBH_4$ in 10 mL methanol and kept for 2 h at room temperature. 5 mL of 2 N H_2SO_4 was added to this reaction mixture which was dissolved in ether and washed with Na_2SO_4. Ether was evaporated to get D-6, m.p. 165°C, $M^+ = 268$ and m.f. $C_{15}H_{24}O_4$.

Permethylation and Hydrolysis of the Glycoside D-1

80 mg of the xanthanolide glycoside D-1 was treated with methyl iodide (10 mL), silver oxide (195 mg) and dimethyl formamide in a 250 mL conical flask at room temperature. The reaction mixture was filtered and the residue washed with dimethyl formamide. The filtrate was evaporated under reduced pressure and the residue so obtained was dissolved in methanol. Removal of the solvent gave a syrupy mass which on hydrolysis with 2% H_2SO_4 gave the aglycone D-2.

The aqueous filtrate was neutralised with $BaCO_3$; the precipitated $BaSO_4$ was filtered off. The filtrate was concentrated and examined by Co-paper chromato-

graphy using BAW (4 : 1 : 5) as solvent system and aniline hydrogen phthalate as spraying reagent.

Peridodate Oxidation of Xanthanolide Glycoside D-1

100 mg of glycoside D-1 was taken in a 100 mL conical flask. 25 mL of sodium metaperiodate was added and the reaction mixture was left for two days. A blank was run simultaneously in the same way. The formic acid liberated and quantity of sodium metaperiodate consumed were estimated quantitatively by the method of Jones.[16]

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