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A new anthraquinone glucoside from the stem bark of *Cassia grandis* Linn.

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Abstract : Keeping in view a great deal of work on various parts of the *Cassia* genus, the work has been undertaken on stem bark of *Cassia grandis* linn. In course of distillation of the parts of *Cassia* genus with ethyl acetate certain compounds we found among which 1,5,8 trihydroxy 6,7 dimethoxy 2-methyl anthraquinone 3-O-β-D(+) glycopyranose was obtained which was established with the standard colour reactions and spectra of the compound.

(Keywords : *Cassia grandis* Linn., stem bark, aglycone, Anthraquinone, glycoside)

Introduction

Many researchers are working on the various parts of *Cassia* genus and. About six hundred species of *Cassia* genus. Many useful compounds have been isolated from this genus. In India fifty genus of *Cassia* have been planted and the workers are working on different parts of these plants. Present work is also a part of such one.

Experimental

Solubility

The compound was soluble in ethyl acetate, benzene, ethanol and methanol, where as insoluble in petroleum ether and slightly soluble in benzene

Glycosidic nature of the compound

About 5 mg of the compound was placed in a test tube containing ethanol and 3-4 drops Conc. H_2SO_4 was added from the side of the test tube slowly. A violet ring formed at the junction of the two liquids, indicating positive Molish test.

Chromatography

Paper Chromatography

Ascending type of paper chromatography was done using whatmann filter paper no. 1 chromatostrips and the following solvent systems.

1. n-butanol : acetic acid : water (4:1:5 v/v)
2. acetic acid : water: Conc. HCl (30:10 v/v)

The developed chromatogram was dried and exposed to the vapors of ammonia, a pink coloured spot appeared.

Thin layer chromatography

It was done on silica gel 'G' plates, after activating the plates at 100°C for 15 minutes in an oven. The following solvent systems were used for developing the plates.

1. Benzene : ethyl acetate :: 5:5 v/v
2. Chloroform : methanol :: 7:3 v/v

A single pink coloured spot was developed with vapour of ammonia.

Hydrolysis of the glycoside and identification of sugar.

About 0.05gm of the compound was dissolved in minimum quantity of ethanol taken in a round bottom flask 20 ml of ethanolic H_2SO_4 (7%) was added and mixture was refluxed for six

hours. The excess of ethanol was distilled off and the contents poured in excess of distilled water (200ml). On cooling a yellowish solid having m.p. 250^0C appeared which was filtered, washed with water and finally crystallized from petroleum ether-ethyl acetate mixture.

Identification of sugar

Since the R_f values of D (+) glucose and D(+) galactose are almost the same (0.18 & 0.16 respectively) hence their benzylamine derivatives were Co-Chromatographed and ninhydrin was used as spraying reagent. D (+) glucose was found to be present, which was further confirmed by Co-Chromatography with an authentic sample of D (+) glucose.

Study of Aglycone
Zinc-dust distillation of the aglycone

The aglycone (0.02 gram) was mixed with one gram of Zn-dust and mixture was shaken in a R.B. flask (25 ml.) having a B_{10} socket. It was fitted with B_{10} quick fit cone of the tube. Suctioned at the other end of the tube by a follow of water (air replaced by nitrogen), the reaction mixture was heated at a high temperature. The distillate was received in flasks. Heating discontinued when issuing of yellow flames from the flasks creased flasks were then disconnected.

The contents of the flasks (Containing ethanol) was then intermixed. Solvent was distilled off. Then a crude product was obtained. It was recystallized from ethanol and m.p. determined. It was found to be 207°C which was confirmed by Co-Chromatorgraphy with an authentic sample of 2-methyl anthracene.

Acetylation

The compound (50 mg) acetylated with acetic anhydride (5ml) in pyridine (2ml) in 100 ml. conical flask using quick fit oppratus and heating on water bath for 30 minutes. On pouring in distilled water 25 ml the product was obtained. Which on crystallization with methanol gave the pure product m.p. 238°C and analysed for acetyl groups.
Acetylated product $C_{17}H_{10}O_8(COCH_3)_4$.

Acetyl percentage

Found	Calcualted
C = 57.85%	C = 58.36%
H = 3.91%	H = 4.28%

Estimation of-OCH_3 group.

The so obtained compound was subjected to Zeisel's method[1] for estimation of methoxyl group which gave the following percentage of methoxyl group.

Found	Calcualted
$-OCH_3 = 17.78\%$	$-OCH_3 = 17.91\%$

Methylation of compound

The compound (50 mg) was dissolved in dry acetone (20 ml) and anhydrous potassium carbonate (2 mg) by usual method. The crude methylated product was obtained and crystallized from methanol (m.p. 207°C)

The product so obtained was again estimated for methoxyl group by Zeisel's method[1] which gave the following percentage of methoxyl group.

Found	Calcualted
46.14%	46.26%

Peroidate oxidation of glycoside

About 0.025 gm of the glycoside dissolved in 50 ml of 50% ethanol and distilled water (25ml). It was treated with 1N sodium meta periodate solution (25ml). A plank experiment was also conducted side by side. Both of these were kept for 48 hours at room temperature. The

periodate consumed and formic acid liberated were determined by titrimetric method. (Jones et al)[2]
For each mole of glycoside
Moles of periodate consumed = 2.14
Moles of formic acid liberated = 1.11

Emulsion Hydrolysis

About 0.023 gm of the glucoside was dissolved in aqueous ethanol (40ml) added emulsion solution to it. The content was heated 40-45^{0}C for four days. The solution was extracted with ethyl acetate and remaining hydrolysate was concentrated in the rotavapour. The syrup so obtained reduced fehling solution and gave clear spot (paper chromatography R_f (0.18) showing the presence of D(+) glucose in hydrolysate.

Results and Discussions

Stem bark of *Cassia grandis* was collected and dried in shade. The small pieces of the bark (about 2.5 kgs) put in soxhlet for extraction with ethyl acetate. The extract obtained was purified through column chromatography. The pure extract was subjected to various processes to detect the presence of constituents. It was found that its molecular formula was $C_{23}H_{24}O_{13}$.

The above compound when subjected to Molisch's test it formed violet ring at the junction indicating positive Molisch's test. The compound when treated with aniline hydrogen phthalate. It did not give the characteristic colour showing that reducing group C_1 of the sugar is not free and involved in glycoside formation. The compound was subjected to acid hydrolysis with 7% H_2SO_4 solution. It produce an aglycone and a free sugar in hydrolysate.

The hydrolysate on neutralization and subsequent concentration under reduced pressure gave a glycone (yellow) and the hydrolysate gave the test of sugar by forming an osazone.

The study of the aglycone

The aglycone was pale yellow in colour and its m.p. was found to be 250^{0}C purity and homogeneity was tested by T.L.C. and P.C. Afterwords the aglycone was subjected to various standard colour reactions for study. Its molecular formula was found to be $C_{17}H_{14}O_8$.

It responded to following reactions

It got reduced with Zn-dust/dil. HCl (Karrer)[3] and Zn-dust/NaOH (Houben)[4]

(a) Alcoholic Solution of the aglycone gave red colour which decolourised gradually (Thomson)[5].
(b) The aglycone when treated with 5N NaOH it produced red colour that intensified on heating (Feigl)[6].
(c) The aglycone in benzene when treated with dil. NaOH the aqueous layer turned red and benzene layer lost its colour (Robinson)[7].

The above colour reactions showed it to be an anthraquinone derivative.

The Basic Skeleton

The aglycone was distilled with zinc dust. It produced 2-methyl anthracene and its m.p. was 207^{0}C. This was supported by its peak 2894cm^{-1} in IR. UV of the aglycone showed peak in alcohol at $\lambda_{max.}^{EtOH}$ 484nm in the visible range.

This hinted that the aglycone had four hydroxyl groups. The aglycone when treated with $FeCl_3$ solution it produced green colour that showed that the hydroxyl groups were phenolic in nature. This was supported by IR peaks at 3400cm^{-1}. It produced no red colour in cerric ammonium nitrate solution which showed the absence of alcoholic (–OH) group.

The aglycone was tested for methoxy group by Zeisel's method. It responded positively showing the presence of methoxy group in the molecule. Thus the aglycone contains both phenolic (–OH) & methoxy group.

Detection of position of (–OH) groups

(a) The observed strong UV maxima at $\lambda_{max.}^{EtOH}$ 484nm hinted that it had at least three α hydroxyl groups (Shibata et al)[8].

(b) The aglycone was soluble in 5%. Na_2CO_3 solution showing that it has β-(–OH) group.

(c) The alcoholic solution of the aglycone was treated with alcoholic solution of $CuSO_4$. Which formed a green complex showing the presence of –OH group adjacent to > C=O group (Somogyi)[9].

(d) It produced red colour with KOH (10%) and formamide showing the presence of 1.8 dihydroxy system in the aglycose.

This was also supported by the IR peaks at 1670cm^{-1} and 1620cm^{-1} for singly and doubly chelated > C=O group.

(e) It produced red colour with cone. H_2SO_4 showing the presence of 1,5-dihydroxy system in the aglycone (Briggs et al)[10]. Thus the three hydroxyl groups were shown to be at positions 1,5 & 8 in the aglycone. As the aglycone was soluble in 5% Na_2CO_3 solution so the fourth (–OH) group must be at any of the β–positions 3, 6 or 7.

(f) A chromatogram of the aglycone was developed and sported with 5% methanolic magnesium acetate. A pink colour developed indicating the presence of dihydroxy system in the molecule (Shibata *et al*)[8].

Thus all the four –OH groups were fixed in the aglycone i.e. at positions 1,3,5 & 8.

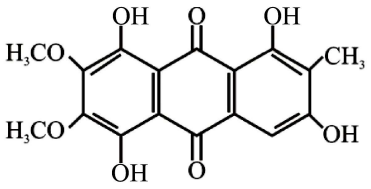
Position of methoxy group

1. It IR peaks at 2860cm^{-1} and 1160cm^{-1} showed the presence of methoxyl group in the aglycone.

2. The aglycone on treatment with Conc. H_2SO_4 80% at 100^{0}C for few minutes, a dark red colour was not obtained showing the absence of α-methoxyl group in the aglycone. So the methoxy group must be at any of the β-positions.

3. Number of methoxy group was estimated by Zeisel's method. It showed the presence of two $–OCH_3$ groups in the aglycone.

On the basis of above tests it was concluded that the structure of the aglycone was 1,3,5,8 tetrahydroxy 6,7-dimethoxy-2-methyl anthraquinone.



Now the position of glycoside linkage was determined by the following tests.

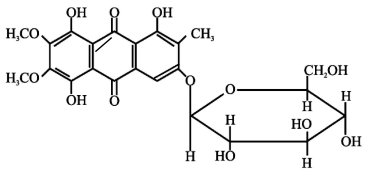
The chromatogram of the glycoside was developed and sprayed with 5% Methanolic magnesium acetate, on heating the chromatogram at 90^{0}C for a few minutes a pink colour was obtained showing the absence of 1,3–dihydroxy system in the glycoside where as the aglycone responded it positively showing that the sugar was linked to aglycone with 3 or 4 of the aglycone.

The glycoside when subjected to the periodic oxidation, it consumed two moles of periodate per mole of the glycoside and produced one mole of formic acid. Thus the sugar is present in paranose form.

Since the glycoside is non–reducing the glucose moiety must be linked to the aglycone nucleus through C_1 of reducing sugar. The glycoside was hydrolysed by enzyme emulsion, so its linkage must be β in nature.

The ozone of the sugar was similar to that of glucose. Hence that sugar moiety was glucose.

On the basis of the experiments, it was concluded that the glycoside was 1,5,8 trihydroxy 6,7 dimethoxy-2-methyl 3-O-β-D(+) glucopyranose.



1,5,8-trihydroxy 6,7-dimethoxy-3-O-β-D(+) glucopyranose

Conclusion

It is ovious that the reports of finding different anthraquinone derivatives and other related compound are true and further works are required on this genus.

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