Phytochemistry, Vol. 20, No. 4, pp. 860–861, 1981.
Printed in Great Britain.

0031–9422/81/040860–02 $02.00/0

AN ELLAGIC ACID GLYCOSIDE FROM THE PODS OF *PROSOPIS JULIFLORA*

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(*Received* 30 *May* 1980)

Key Word Index—*Prosopis juliflora*; Leguminosae; ellagic acid 4-*O*-α-L-rhamnosylgentiobioside.

Abstract—From the fresh pods of *Prosopis juliflora*, a new glycoside has been isolated and characterized as ellagic acid 4-*O*-α-L-rhamnosylgentiobioside.

Prosopis juliflora DC (Leguminosae) is an economically important plant of medicinal value [1, 2], well known for its antibiotic and antibacterial properties [3]. Polyphenols and tannins [4] and some phenolic constituents of the stem bark [5] and pods [6] have been reported. The present paper describes the characterization of a new glycoside from an ethanolic extract of the fresh pods.

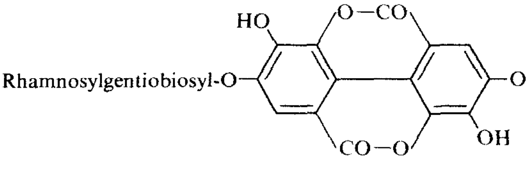
This compound was found to be a non-reducing glycoside. Colour reactions with ferric chloride and alkali indicated its phenolic nature. Preliminary diagnostic tests, including a positive Greissmeyer reaction, suggested that it was an ellagic acid derivative and this was further substantiated by UV and IR spectral analysis. Its IR spectrum was very similar to that of ellagic acid gentiobioside. On acid hydrolysis, it gave ellagic acid, rhamnose and glucose. Ellagic acid was identified by mp, colour tests, solubility, spectral analysis and co-chromatography with an authentic synthetic sample prepared by persulphate oxidation of gallic acid. Sugars were identified by co-chromatography with authentic samples and preparation of their corresponding osazones. Quantitative acid hydrolysis suggested the presence of three mol of reducing sugar per mol of ellagic acid, which was confirmed by elemental analysis of the glycoside and its derivatives.

As the glycoside was unaffected by aqueous alkali, this eliminated the possibility of ester linkage to a carboxylic acid grouping in hexahydroxydiphenic acid. The position of the sugar unit was determined with the help of spectral shifts with specific reagents [8], and methylation and hydrolysis of the glycoside. A bathochromic shift of 53 nm with sodium ethylate indicated the presence of at least one free hydroxyl either at the 3,3′ or 4,4′ position. A bathochromic shift (21 nm) with sodium acetate suggested the presence of at least one strongly acidic hydroxyl, i.e. in the 3 or 3′ position. The exact position of sugar unit was determined by methylation of the glycoside with diazomethane followed by acid hydrolysis to give 3,3′,4′-trimethyl ellagic acid, which gave a monoacetate. This confirmed that all the three sugar units are linked as a trisaccharide at position 4 of ellagic acid.

The molar ratio of rhamnose to glucose was ascertained as 1:2 by comparative PC using artificial mixtures with different molar proportions of the two sugars along with the hydrolysate. Rhamnose was shown to be the terminal sugar as it appeared before glucose during mild acid hydrolysis (1% HCl) of the glycoside.

In order to establish the position of the intersugar linkages in the carbohydrate moiety, the glycoside was completely methylated and then acid hydrolysed to give 2,3,4-tri-*O*-methyl glucose, and 2,3,4-tri-*O*-methyl rhamnose, identified by their R_G values. This suggested the presence of a gentiobiose unit, linked to rhamnose by a (1 → 6) linkage and also indicated the pyranose form for all three sugar units. Further information was provided by periodate oxidation of the diazomethane methylated glycoside. The liberation of 3 mol of formic acid, with consumption of 6 mol of sodium metaperiodate supported the pyranose ring structure for all the three sugar moieties.

The stereochemistry of the two intersugar linkages and one glycoside linkage was determined by enzyme hydrolysis. When takadiastase was used, rhamnose was the only free sugar detected and ellagic acid gentiobioside was extracted from the solution with ethyl acetate, mp 246° (lit. 248–50°) [7] and gave a superimposable IR spectrum with that of a natural sample reported earlier [7]. This gentiobioside of ellagic acid was completely hydrolysed to ellagic acid and glucose by almond emulsin, confirming both the glycosidic linkage and the intersugar linkage between the two glucose units to be β. The evidence cited above for the nature of the trisaccharide unit was in agreement with ^{1}H NMR data for the glycoside. Thus, the glycoside is characterized as ellagic acid 4-*O*-α-L-rhamnosylgentiobioside **I**.



I

EXPERIMENTAL

Material. Plant material was collected locally and identified by the Botanical Survey of India, Allahabad branch.

Extraction. Fresh pods and seeds (2 kg) were extracted with boiling EtOH (10 l.). On concn of the EtOH extract a highly viscous concentrate was obtained (200 ml), excess EtOH added and the resulting ppt filtered. The filtrate was concd and extracted with petrol, C_6H_6 and EtOAc. The remaining mother liquor after concn was macerated with Me_2CO to yield **1**, a homogeneous compound which crystallized from Me_2CO–Et_2O as light buff coloured prisms, mp 243–5°, R_f 0.14 (solvent: BAW (4:1:5), spray: ferric chloride (Found: C, 49.15; H, 4.61. $C_{32}H_{36}O_{22}$ requires: C, 49.74, H, 4.66%.) UV: $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 251, +NaOAc 272, +NaOEt 284, IR: $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3440, 1735, 1720, 1625, 1560, 1540, 1480, 1450, 1380, 1340, 1200, 1120, 1040 and 940. ^1H NMR (δ, 60 MHz, Me_2CO d-6): 1.2 (3H, *s*, C-6, Rha); 3.00 (16H, *s*, sugar protons), 4.9 (2H, *s*, C-1″, Glc), 5.2 (1H, *s*, C-1″, Rha), 7.3 (2H, *s*, Ar-proton).

Acid hydrolysis. **I** (0.02 g) was refluxed with aq. H_2SO_4 (7%, 3 ml) for 2.5 hr, the soln cooled and the ppt removed and crystallized from EtOH containing traces of C_5H_5N, mp > 360° (lit. > 360°); UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 254 (lit. 255); IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3340 (–OH), 1740, 1720, 1620, 1520, 1430, 1360, 1220, 1190, 1100, 1060, 920 and 758. The centrifugate was neutralized with $BaCO_3$, concd and the resulting syrup gave glucose and rhamnose.

Quantitative acid hydrolysis. **I** (0.1 g) was refluxed with aq. H_2SO_4 (7%, 3 ml) for 2.5 hr. The ppt was centrifuged, washed and weighed. Filtrate and washings were collected, neutralized with $BaCO_3$, made up to 25 ml and sugars estimated by the colorimetric method of Folin and Wu [9]. (Found: ellagic acid, 38.20; reducing sugar 61.10. $C_{32}H_{36}O_{22}$ requires: ellagic acid, 38.25; reducing sugar 61.93%.)

Methylation, acid hydrolysis and acetylation. To **I** (0.1 g) in Me_2CO (5 ml) excess CH_2N_2 was added, kept for 24 hr in the cold and excess CH_2N_2 removed, the solvent distilled off and the resulting syrup crystallized from dry Et_2O, mp 211°. (Found: —OMe, 11.09, $C_{32}H_{33}O_{19}(OMe)_3$, requires: —OMe, 11.95%.) Acid hydrolysis of the methylated glycoside (0.05 g) with aq. H_2SO_4 (7%, 3 ml) yielded a yellow ppt which crystallized from Me_2CO–Et_2O as yellow prisms, mp 286° (lit. 288–9°). The acetate crystallized from dioxane–petrol as pale yellow crystals, mp 262° (lit. 264–5°).

Hydrolysis with takadiastase. To **I** (0.04 g) in EtOH (20 ml) diastase soln (1 g in 25 ml) was added. The reaction mixture was left at 20–25° for 3 days, the soln centrifuged and the decanted conc hydrolysate chromatographed on PC, R_f 0.37 (solvent: BAW; spray: aniline hydrogen phthalate). Co-PC with authentic rhamnose gave R_f 0.36. The residue (ellagic acid gentiobioside) obtained on centrifugation crystallized from EtOH–C_5H_5N. UV: $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 251 (lit. 252); IR: $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3500, 3320, 1750, 1720, 1625, 1560, 1550, 1520, 1450, 1340, 1200, 1110 and 1045.

Periodate oxidation. To methylated **I** (0.02 g) dissolved in EtOH (20 ml) $NaIO_4$ soln (20 ml, 0.01 M) was added and the mixture kept at 25° for 48 hr. A blank was also run. The periodate consumed and the formic acid liberated were estimated by the titrimetric method of Hirst and Jones [10]. For each mol of glycoside 6.6 mol of periodate were consumed and 3.1 mol of formic acid produced.

Acknowledgement—One of the authors (S. M.) is grateful to the CSIR, New Delhi, India for financial support.

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