petrol (bp 40–60°); from 120 g *C. integristipula*, 1.3 g of the extract was obtained, from 57 g *C. neesiana*, 1.0 g, and from 54 g *C. sphagnicola*, 1.2 g. The extract of *C. integristipula* and *C. neesiana* was chromatographed on Si gel columns. Using petrol with 2% benzene as eluant, crystalline compounds (77 and/or 49 mg) mp 95–96° were isolated, identical according to their IR (KBr), MS and NMR data. Using prep. HPLC, 40 mg of the same compound were isolated from *C. sphagnicola*. All spectral data of the three mentioned products are in accordance with those published by Huneck [5].

Alkaline hydrolysis. The crystalline ester (34.1 mg) originally, for example, from *C. integristipula*, was hydrolysed under reflux in ethanolic KOH (68.2 mg in 6 ml) for 3 hr. After the usual work-up, campesterol (13.0 mg), mp 156–157°, $[\alpha]_D^{25}$ −32.3°, (*c* 0.14) identical with an authentic specimen, was isolated. In the GC analysis an admixture of about 3% sitosterol was observed, and it was identified by co-chromatography with an authentic specimen. The same findings were confirmed by MS analysis. The acid originating from the hydrolysis (12.0 mg, mp 80°) was identified as behenic acid as the methyl ester and compared by GC with an authentic specimen.

Reduction and acetylation [8]. Campesteryl behenate (20.6 mg) was reduced in EtOAc (6.2 ml) soln of $LiAlH_4$ (51 mg), and then acetylated with Ac_2O. After the usual work up campesterol acetate (7.5 mg, mp 136°) and docosanol acetate (6.4 mg, mp 60–63°, ref. [10] 64–65°) were identified.

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ELLAGIC ACID 4-*O*-RUTINOSIDE FROM PODS OF *PROSOPIS JULIFLORA*

S. MALHOTRA and K. MISRA

Chemical Laboratories, University of Allahabad, Allahabad 211002, India

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Key Word Index—*Prosopis juliflora*; Leguminosae; pods; ellagic acid 4-*O*-rutinoside.

Abstract—From the pods of *Prosopis juliflora* a new glycoside, ellagic acid 4-*O*-rutinoside, has been characterized.

In our previous communications [1, 2] we have reported the presence of ellagic acid 4-*O*-α-L-rhamnosylgentiobioside in the pods [1] and 3,3-di-*O*-methylellagic acid 4-*O*-α-L-rhamnopyranoside in the roots [2] of *Prosopis juliflora*. We now report the isolation and characterization of another ellagic acid glycoside (**1**) from the pods of the same plant.

Compound **1** was found to be a non-reducing glycoside, which gave a dark bluish green precipitate with ferric chloride and yellow colour with alkali indicating its phenolic nature. Preliminary diagnostic tests, including a positive Greissmeyer reaction, suggested that it was an ellagic acid derivative. Absorption maxima at 238 nm and strong IR peaks at 3440 (—OH) and 1725 cm^{-1} (lactone) were similar to other ellagic acid derivatives [1–5]. On acid hydrolysis it gave ellagic acid [mp, colour tests, solubility UV, IR and co-paper chromatography (co-PC)] rhamnose and D-glucose. The sugars were identified by co-PC with authentic samples and preparation of their corresponding osazone derivatives. Quantitative acid hydrolysis showed the presence of 2 mol reducing sugar/mol ellagic acid and was further substantiated by elemental analysis of the glycoside and its derivatives.

The glycoside **1** was unaffected by aqueous alkali, eliminating the possibility of its being a sugar ester involving bidentate ester linkages with hexahydroxydiphenic acid. A bathochromic shift of 47 nm with sodium ethylate indicated the presence of at least one free

hydroxyl either at the 3,3′ or the 4,4′ position. A bathochromic shift of 36 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 the sugar unit was determined by methylation of **1** with diazomethane followed by acid hydrolysis when 3,3′,4-trimethylellagic acid, mp 286° (lit. 288–289°), was obtained, which gave a monoacetate, mp 262° (lit. 264°). This confirmed that both the sugar units are linked at position 4 of ellagic acid as a disaccharide. In the disaccharide unit, glucose and rhamnose were present in molar ratio of 1:1 as indicated by co-PC. Rhamnose was found to be the terminal sugar as it appeared first during the mild acid hydrolysis of the glycoside, followed later by glucose.

As the glycoside is non-reducing, the reducing groups of both sugars must be involved in linking. Thus, C-1 of the glucose unit must be linked at position 4 of ellagic acid and C-1 of the rhamnose must be involved in an intersugar linkage with a hydroxyl of the glucose. The structure was finally established by permethylation of **1** followed by acid hydrolysis, which gave 2,3,4-tri-*O*-methyl rhamnose and 2,3,4-tri-*O*-methyl glucose, identified by their R_G values [6]. This confirmed the (1 → 6) inter-sugar linkage. These results also confirmed a pyranose ring structure for both sugars as the hydroxyls at position-4 in both sugars were methylated. Further, structural information was provided by periodate oxidation of the diazomethane methylated glycoside. The liberation of 2 mol of formic acid with consumption of 4 mol of sodium metaperiodate supported the pyranose form for both sugars.

The stereochemical nature of the inter-sugar as well as the glycosidic linkages was established by enzymic hydrolysis of **1**. Thus, with takadiastase only rhamnose and the partially hydrolysed glycoside, which was completely hydrolysed with almond emulsin, were found. This confirmed the inter-sugar linkage as α and the glycosidic linkage as β in nature. The evidences cited above were confirmed by ^{1}H NMR data for **1**. Thus, **1** is identified as ellagic acid 4-*O*-rutinoside.

EXPERIMENTAL

Plant material. The plant material was collected locally and identified by the Botanical Survey of Allahabad, Allahabad Branch, India.

Chromatography. R_f values are for ascending PC except for the sugar, the solvents being (a) BAW (4:1:5); (b) 5% HOAc and (c) *n*-BuOH–EtOH–H_2O (5:1:4).

Extraction. Fresh pods (3 kg), including the seeds, were crushed and extrd with boiling EtOH (5 × 2 l). On concn of the EtOH extract under red. pres. a highly viscous concentrate was obtained (200 ml) to which excess EtOH was added (2 × 5 l.) and the resulting ppt removed. The filtrate was concd and extracted with petrol (2 × 4 l.) to give an amorphous residue, which was filtered, washed with petrol and crystallized from dry Me_2CO–Et_2O as light orange-pink semimicro-crystals, mp 253° (d) $[\alpha]_{D(\text{pyridine})}^{25°}$ −41°, R_f 0.12 and 0.30 (solvents: a and b; spray: alc. $FeCl_3$). Found: C, 50.90; H, 4.10 calc. for $C_{26}H_{26}O_{17}$ C, 51.14 and H, 4.26%. λ_{max}^{EtOH} nm: 238, 267; + NaOAc: 274; NaOEt: 285, 314. ν_{max}^{KBr} cm^{-1}: 3950, 25, 1700, 1610, 1580, 1500, 1450, 1375, 1200, 1120, 1060, 980, 940 and 820 ^{1}H NMR (60 MHz, Me_2CO-d_6) δ: 1.3 (*s*, 3 H-Me Rha); 3.10 (*s*, 10 H, most of sugar protons), 4.9 (*s*, 1 H, C-1 of Glc); 5.1 (*s*, 1 H, C-1 of Rha); 7.2 (*br s*, 2 H, Ar protons).

Quantitative acid hydrolysis. **1** (0.01 g) was refluxed with aq. H_2SO_4 (7%, 3 ml) for 2.5 hr, cooled, centrifuged and ppt. dried and weighed. The filtrate and washings were collected and neutralized with $BaCO_3$, made up to 25 ml, and sugar estimated by the colorimetric method of Folin and Wu [7]. Found: ellagic acid 48.1, reducing sugar: 51.5 calc. for $C_{26}H_{26}O_{17}$, Ellagic acid 49.30 and reducing sugar 51.5%.

For other experimental details see ref. [1, 2].

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