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Note

Ellagic acid derivatives from the leaves of *Eugenia jambos* Linn†

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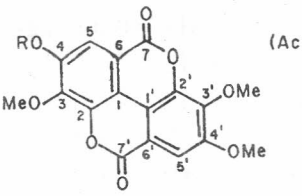
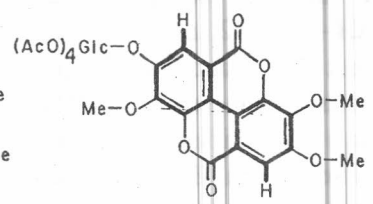
From the methanolic extract of the leaves of *Eugenia jambos*, a new ellagic acid derivative, 3,3′,4′-tri-*O*-methylellagic acid-4-*O*-β-D-glucopyranoside has been isolated along with 3,3′,4′-tri-*O*-methylellagic acid. Its structure has been elucidated mainly on the basis of 2D NMR spectral analyses and chemical correlation.

Ellagic acid is a common unit present in plant tannins. Similar phenolic substances, known as nasutins, are also found in several Australian species of the termite genus *Nasutitermes*[1,2]. However, methylated ellagic acids are rarely encountered in plant sources. During our chemical investigation on the leaves of *Eugenia jambos* Linn., a plant used in various ailments in the Indian system of medicine[3], a sparingly soluble solid was obtained from its methanolic extract. The solid could not be purified as such because of its very poor solubility. However, chromatographic purification through its acetate led to the isolation of two ellagic acid derivatives **1** and **2** which were characterized mainly on the basis of 2D NMR spectral analyses and chemical correlation. We report herein the structure elucidation of **1** and **2**.

Compound **1**, mp 258-260°C, $[\alpha]_D$ -26.6°, did not show the molecular ion in its EIMS, though it exhibited two intense fragment ions at *m/z* 331 and 343 for a peracetylated hexose unit and the aglycone moiety respectively. The compound on hydrolysis with 5% HCl yielded the aglycone **3** which on acetylation with Ac_2O-pyridine at room temperature furnished the monoacetate **2**



1 R = Tetraacetyl glucosyl
2 R = Ac
3 R = H



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establishing the correlation between **1** and **2**. On the other hand, the crude solid obtained from the methanolic extract on hydrolysis with 5% HCl yielded D-glucose (PC). The ^{1}H NMR spectrum of **1** displayed, besides the signals for four acetoxyl methyl groups and other carbinyl protons of the peracetylated glucosyl moiety (*vide* Experimental), three three-proton singlets at δ 4.047, 4.143 and 4.221 for three aromatic OMe groups, and two one-proton singlets at δ 7.689 and 7.912 for two isolated aromatic CH. Its ^{13}C NMR spectrum (**Table I**) displayed signals for 31 carbons, of which 14 belong to peracetylated glucosyl unit (4 acetoxyl CH_3, one CH_2, 5 CH and 4 acetoxyl

Table I—^{13}C Chemical shifts* (100 MHz, δ TMS) of **1** and **2**

Carbon	1	2	Carbon	1	2
1	115.29	117.94	4′–OMe	56.86	56.85
2	141.54	142.32	1″	100.11	–
3	143.15	145.04	2″	70.84	–
4	151.51	144.83	3″	72.35	–
5	113.69	120.20	4″	68.29	–
6	112.48	113.08	5″	72.70	–
7	158.32	158.35[a]	6″	62.05	–
1′	112.66	113.20	$OCOCH_3$	20.62	20.37
2′	141.35	141.72		20.62	–
3′	141.93	142.52		20.71	–
4′	154.90	155.83		20.71	–
5′	107.99	108.59	$COCH_3$	169.31	168.89
6′	112.95	113.90		169.49	–
7′	158.65	158.38[a]		170.13	–
3–OMe	62.25	61.68[b]		170.94	–
3′–OMe	61.98	61.88[b]			

*Spectra of **1** and **2** were recorded in $CDCl_3$ and pyridine-d_5 respectively. Assignments in case of **1** were based on HSQC and HMBC spectral analyses.

[a,b]Values in a vertical column may be interchanged.

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carbonyl carbons) and remaining 17 carbons to the aglycone moiety (3 aromatic OMe, 2 aromatic CH and 12 quaternary aromatic carbons). The IR spectrum of **1** showed absorption bands for OAc and aromatic δ-lactone at 1743 and 1607 cm^{-1} respectively. A literature survey showed that the carbonyl carbon of ellagic acid, an aromatic δ-lactone, resonates at considerably up-field[4] between δ 158.0-160.0. Since in the ^{13}C NMR spectrum of 1, two quaternary carbon signals appeared at δ 158.32 and 158.65, and also only two aromatic CH carbons are present in the molecule, an ellagic acid type structure was considered for the compound. Although ellagic acid is a symmetrical molecule, because of the presence of three OMe and one acetylated glucosyloxy moiety, usual symmetry was lost thereby showing separate signals for each individual carbons in its ^{13}C NMR spectrum. The observed M^+ at *m/z* 386 in the EIMS of **2** could also be accounted for on the basis of monoacetyl-tri-*O*-methylellagic acid structure for **2**.

In order to find out the locations of three OMe groups and one sugar unit in the molecule, compound **1** was subjected to detailed 2D NMR, particularly HSQC and HMBC spectral analyses, and the one-bond and multiple-bond ^1H-^{13}C correlation data (cf.**Table II**). It can be seen therein that while H-5 showed two-, three- and even four-bond correlations with C-1, C-2, C-3, C-4, C-6, C-7 and C-1′, H-5′ was found to be correlated with C-1′, C-2′, C-3′, C-4′, C-6′, C-7′ and C-1 thereby establishing the part structure shown by heavy line in **4**. The three-bond correlations of the two OMe proton signals at δ 4.221 and 4.047 with C-3′ (δ 141.93) and C-4′ (δ 154.90) indicated that the remaining OMe group and the peracetylglucosyloxy moiety must be located at C-3 and C-4. The three-bond correlation of the anomeric proton (H-1″, δ 5.180) with C-4 (δ 151.51) and that of the OMe proton signal (δ 4.143) with C-3 (δ 143.15) clearly demonstrated the location of the peracetyl-glucosyloxy moiety and the OMe group at C-4 and C-3 respectively. This assignment was further corroborated by the up-field shift of C-3 and C-5 (β-carbons) of **1** by ~ **2** and 7 ppm, and deshielding of its C-4 (α-carbon) signal by ~ 7 ppm as compared to those of 2 (**Table I**).

On the basis of the above observations, **1** and **2** could be represented as 3,3′-4′-tri-*O*-methylellagic acid-4-*O*-β-D-tetraacetylglucopyranoside and 4-*O*-acetyl-3,3′,4′-tri-*O*-methylellagic acid respectively.

It may be mentioned that 3,3′,4′-tri-*O*-methylellagic acid **3** was earlier reported from some termite species[2]. However, this seems to be the first report on the isolation of **3** or its β-D-glucopyranoside from a plant source.

Experimental Section

General. Melting points are uncorrected. 1D and 2D NMR spectra were recorded on a Bruker DRX 400 (400 MHz) instrument in $CDCl_3$ with TMS as internal standard. EIMS were taken on a JEOL HX-110 instrument at 30 eV.

Table II—One-bond and multiple-bond ^1H-^{13}C correlation data of **1**

δ_H	One-bond correlation δ_C	Multiple-bond correlation δ_c			
7.912 (H-5)	113.69 (C-5)	112.48 (C-6)	112.66 (C-1′)	115.29 (C-1)	141.54 (C-2)
		143.15 (C-3)	151.51 (C-4)	158.32 (C-7)	
7.689 (H-5′)	107.99 (C-5′)	112.66 (C-1′)	112,95 (C-6′)	115.29 (C-1)	141.35 (C-2′)
		141.93 (C-3′)	154.90 (C-4′)	158.65 (C-7′)	
4.047(CH_3O-4′)	56.86 (CH_3O-4′)	154.90 (C-4′)			
4.143(CH_3O-3)	62.25 (CH_3O-3)	143.15 (C-3)			
4.221 (CH_3O-3′)	61.98 (CH_3O-3′)	141.93 (C-3′)			
5.180 (H-1″)	100.11 (C-1″)	70.84 (C-2″)	72.70 (C-5″)	151.51 (C-4)	
5.414 (H-2″)	70.84 (C-2″)	72.35 (C-3″)	100.11 (C-1″)	169.31 (OAc)	
5.353 (H-3″)	72.35 (C-3″)	70.84 (C-2″)	00.11 (C-1″)	170.13 (OAc)	
5.166 (H-4″)	69.29 (C-4″)	62.05 (C-6″)	72.70 (C-5″)	169.45 (OAc)	
4.007 (H-5″)	72.70 (C-5″)	62.05 (C-6″)	68.29 (C-4″)	72.35 (C-3″)	100.11 (C-1″)
4.265 (H_2-6″)	62.05 (C-6″)	68.29 (C-4″)	72.70 (C-5″)	170.94 (OAc)	

Plant material. The leaves of *Eugenia jambos* were collected from M/s United Chemical and Allied Products, 10, Clive Row, Calcutta 700 001. and a voucher specimen is available in the herbarium of the company.

Extraction and isolation. The defatted leaves (2.0 kg) were extracted with MeOH-$CHCl_3$ (1:1) at room temperature. The extract was concentrated (250 mL). Water (1L) and $CHCl_3$ (500 mL) were added to the concentrate and the mixture was shaken. The insoluble solid (0.6 g) was filtered out. The solid (0.275 g) was acetylated with Ac_2O-pyridine at 100°C for 3 hr. After usual work-up, the crude product was chromatographed over silica gel column (10 g) to get pure **1** (0.14 g) and **2** (30 mg).

3, 3′, 4′-Tri-*O*-methylellagic acid-4-*O*-β-D-tetraacetyl-glucopyranoside 1. Crystallised from EtOAc-$CHCl_3$ as fine needles, mp 258-260°C, $[\alpha]_D$ -26.6° (*c*, 0.4, $CHCl_3$); IR (KBr): 1743, 1226 (OAc), 1607 cm^{-1} (aromatic lactone); ^1H NMR δ 2.064, 2.084, 2.117, 2.191 (3H each, s, $OCOCH_3$ × 4), 4.007 (1H, m, H-5″), 4.047 (3H, s, MeO-4′), 4.143 (3H, s, MeO-3), 4.221 (3H, *s*, MeO-3′), 4.265 (1H, d, *J*=4.3 Hz, H_2-6″), 5.166 (1H, t, *J*=9.6 Hz, H-4″), 5.180 (1H, d, *J*=7.6 Hz, H-1″), 5.353 (1H, *t*, *J*=9.7 Hz, H-3″), 5.414 (1H, t, *J*=9.5 Hz, H-2″), 7.689 (1H, s, H-5′), 7.912 (1H, s, H-5); ^{13}C NMR: δ. **Table I**; EIMS: *m/z* 344 (35%), 343 (36), 331 (28), 271 (17), 169 (100), 109 (39).

4-*O*-Acetyl-3,3′,4′ -tri-*O*-methylellagic acid 2. Crystal-lised from EtOAc-$CHCl_3$ as shining flakes, mp 268-270°C; IR (KBr): 1740, 1252 (OAc), 1611 cm^{-1} (aromatic lactone); ^1H NMR: δ 2.395 (3H, s, $OCOCH_3$), 4.055 (3H, s, MeO-4′), 4.230 (3H, s, MeO-3′), 4.290 (3H, s, MeO-3), 7.735 (1H, s, H-5′), 7.910 (1H, s, H-5); ^{13}C NMR: δ **Table I**; EIMS: *m/z* 386 (M^+, 12%), 344 (100), 328 (19), 169 (15), 145 (8), 109 (7).

Acid hydrolysis of 1. A suspension of **1** (15 mg) in 5% methanolic HCl (10 mL) was refluxed for 2 hr. Most of the MeOH was removed under reduced pressure and then water (10 mL) was added to the concentrate. The separated solid was filtered, washed with water and dried to get the crude **3**. It was then acetylated with Ac_2O-pyridine at room temperature for 24 hr. Usual work-up gave a solid which on purification by chromatography over silica gel and crystallisation from EtOAc-$CHCl_3$ furnished **2** (6 mg).

References

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